(19) Europäisches Patentamt
European Patent Office
Offic européen des br v ts

(11) EP 0 491 031 B1

B17

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
01.05.1996 Bulletin 1996/18

(21) Application number: **91912863.7**

(22) Date of filing: **05.07.1991**

(51) Int Cl.[6]: **C12P 21/08**, A61K 39/395, C12N 15/13

(86) International application number:
PCT/GB91/01108

(87) International publication number:
WO 92/01059 (23.01.1992 Gazette 1992/03)

(54) CDR GRAFTED ANTI-CEA ANTIBODIES AND THEIR PRODUCTION

CDR-GEPFROPFTE ANTI-CEA-ANTIKÖRPER UND IHRE HERSTELLUNG

ANTICORPS DIRIGES CONTRE L'ANTIGENE CARCINO-EMBRYONNAIRE (CEA) ET A GREFFE DE ZONES DETERMINANT LA COMPLEMENTARITE (CDR) ET PRODUCTION DE CES ANTICORPS

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(30) Priority: **05.07.1990 GB 9014932**
21.12.1990 WOPCT/GB90/02017

(43) Date of publication of application:
24.06.1992 Bulletin 1992/26

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EP 0 491 031 B1

Description

FIELD OF THE INVENTION

The present invention relates to humanised antibody molecules (HAMs) having specificity for Carcinoembryonic Antigen (CEA) and to processes for their production using recombinant DNA technology.

The term "humanised antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, the remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable regions fused onto human constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate human framework regions in the variable domains. The abbreviation "MAb" is used to indicate a monoclonal antibody.

In the description, reference is made to publications by number, and these publications are listed in numerical order at the end of the description.

BACKGROUND OF THE INVENTION

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the outer end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies of defined specificity (1). However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. The resultant MAbs are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness.

Therefore proposals have been made for making non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs related to production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody are fused to constant domains derived from a second antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc.), EP-A-0171496 (Res. Dev. Corp. Japan), EP-A-0173494 (Stanford University), and EP-A-0194276 (Celltech Limited). The Celltech EP 0194276 application discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. It also describes the production of an antibody molecule comprising the variable domains of a mouse MAb, the CHI and CL domains of a human immunoglobulin, and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

Subsequently a number of further patent applications have been published relating to chimeric antibodies, including tumour specific chimeric antibodies (e.g. WO 87/02671, Int. Gen. Eng. Inc.; EP 0256654, Centocor; EP 0266663, Int. Gen. Eng. Inc. & Oncogen; WO 89/00999, Int. Gen. Eng. Inc. and EP 0332424, Hybritech Inc.). The Genentech (EP0125023) and Hybritech (EP0332424) patent application relate to anti-carcinoembryonic antigen (anti-CEA) chimeric antibodies.

Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete variable region domains. Thus such humanised antibodies may elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 2)].

In an alternative approach, described in EP-A-02394000 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. Such CDR-grafted humanised antibodies are less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the lower proportion of non-human amino acid sequence which they contain. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable regions.

The earliest work on CDR-grafted humanised MAbs was carried out on MAbs recognising synthetic antigens, such

as the NP or NIP antigens. However, recently examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T cells respectively were humanised have been described by Verhoeyen et al (3) and Riechmann et al (4). The preparation of the CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council). More recently Queen et al (5) have described the preparation of a humanised CDR-grafted antibody that binds to the interleukin 2 receptor.

It has been widely suggested that immunoglobulins, and in particular MAbs, could potentially be very useful in the diagnosis and treatment of cancer (6, 7). There has therefore been much activity in trying to produce immunoglobulins or MAbs directed against tumour-specific antigens. So far, over one hundred MAbs directed against a variety of human carcinomas have been used in various aspects of tumour diagnosis or treatment (8).

There have been a number of papers published concerning the production of chimeric monoclonal antibodies recognising cell surface antigens. For instance, Sahagan et al (9) disclose a genetically engineered murine/human chimeric antibody which retains specificity for a tumour-associated antigen. Also Nishimura et al (10) disclose a recombinant murine/human chimeric monoclonal antibody specific for common acute lymphocytic leukemia antigen.

We have now prepared humanised antibodies to carcinoembryonic antigen derived from the anti-CEA mouse MAb A5B7 (11).

International Patent Application PCT/GB90/02017 (published as W091/09967) relates to the CDR-grafting of antibodies in general and describes, among other things, that antibodies having specificity for cancer markers such as CEA, e.g. the A5B7 monoclonal antibody, have been successfully CDR-grafted according to the procedure described herein.

According to the present invention, there is provided an antibody molecule having specificity for carcinoembryonic antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and heavy chain antigen-binding residues from the mouse monoclonal antibody A5B7, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 23 and/or 24, 26 to 35, 50 to 65, 71 and/or, 73 and 95 to 100 are A5B7 residues.

In a first preferred embodiment, in the antibody molecule residue 24 and additionally residue 25 in the heavy chain are A5B7 residues.

In a second preferred embodiment, in the antibody molecule of the invention residue 94 in the heavy chain may additionally be an A5B7 residue.

In a third preferred embodiment, in the antibody molecule of the invention residues 101 and 102 may additionally be A5B7 residues.

In the antibody molecule of the invention residues 6, 48, 49, 69, 76, 78, 80, 88 and 91 may additionally be A5B7 residues.

In the preferred antibody molecule of the invention the heavy chain framework residues are LAY residues. In this embodiment, preferably residues 1, 24, 48, 49, 72, 73, 76 and 93 in the heavy chain are additionally A5B7 residues. If desired, residues 82b and 86 in the heavy chain may additionally be A5B7 residues.

In another aspect, the present invention provides an antibody molecule having specificity for carcinoembryonic antigen and comprising a composite light chain and a complementary heavy chain, said composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and light chain antigen-binding residues from the mouse monoclonal antibody A5B7, wherein, according to the Kabat numbering system, in said composite light chain, at least residues 24 to 34, 46, 47, 50 to 56 and 91 to 96 are A5B7 residues.

Preferably in the antibody molecule of the first aspect of the invention, the complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and light chain antigen-binding residues from the mouse monoclonal antibody A5B7, wherein, according to the Kabat numbering system, in said composite light chain, at least residues 24 to 34, 46, 47, 50 to 56 and 91 to 96 are A5B7 residues.

In antibody molecules containing a composite light chain, preferably residues 89 to 91 and 97 in the light chain are additionally A5B7 residues.

In such antibody molecules residues 1 to 3, 49, 60, 70, 84, 85 and 87 in the light chain may additionally be A5B7 residues.

Preferably the light chain framework residues are LAY residues. In this case, preferably residues 1 to 4, 21, 71 and 73 in the light chain are additionally A5B7 residues.

The A5B7 MAb is a mouse MAb of the type IgG1-Kappa raised against purified CEA which had been denatured by heating to 85°C for 35 minutes. The A5B7 MAb has been extensively studied at Charing Cross Hospital, London, UK (11). Immunohistochemical studies have demonstrated that the A5B7 MAb reacts with CEA producing tumours. Its distribution is within malignant glands in the cell cytoplasm, at the cell surface and in necrotic debris. However, it shows no significant cross-reactivity with a wide spectrum of normal human tissues. The molecular cloning and sequencing of the A5B7 heavy and light chain cDNAs is described hereinafter and the V_L and V_H cDNA and predicted amino acid sequences are given in Figure 1.

Surprisingly it has been found that humanising the A5B7 MAb, in particular CDR-grafting, does not substantially adversely affect its binding activity, and this produces an antibody molecule which is extremely useful in both therapy and diagnosis of certain carcinomas.

The antibody molecule of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as the Fab, Fab', $(Fab')_2$ or Fv fragment; a single chain antibody fragment, e.g. a single chain Fv; a light chain or heavy chain monomer or dimer; including fragments or analogues of any of these or any other molecule with the same specificity as the A5B7 antibody.

The antibody molecule of the present invention may have attached to it an effector or reporter molecule. For instance, the antibody molecule may have a macrocycle for chelating a heavy metal atom, or a toxin such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an antibody molecule in which the Fc fragment or CH3 or CH4 domain of a complete antibody molecule has been replaced by or has attached thereto by peptide linkage a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

The remaining non-A5B7 immunoglobulin derived parts of the antibody molecule may be derived from any suitable human immunoglobulin. For instance, appropriate variable region framework sequences may be used having regard to the class/type of the A5B7 donor antibody from which the antigen binding regions are derived. Preferably, the type of human framework used is of the same/similar class/type as the donor antibody (A5B7 is IgG1 Kappa). Advantageously, the framework is chosen to maximise/optimise homology with the donor antibody sequence particularly at positions spatially close to or adjacent the CDRs. Examples of human frameworks which may be used to construct CDR-grafted HAMs are LAY, POM, TUR, TEI, KOL, NEWM, REI and EU; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU for both the heavy chain and the light chain. Preferably the LAY framework is used as the human framework for both heavy and light chain variable domains, in view of its high level of homology with A5B7.

Also human constant region domains of the antibody molecule may be selected having regard to the proposed function of the antibody, in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular IgG human constant region domains, especially of the IgG1 and IgG3 isotypes, may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for purposes for which antibody effector functions are not required e.g. for imaging, diagnostic or cytotoxic targeting purposes.

However, the remainder of the antibody molecule need not comprise only protein sequences from human immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a polypeptide effector or reporter molecule.

The antibody molecule of the present invention may be produced by a process comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy or light chain as defined above;

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light or heavy chain;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the antibody molecule.

The cell line may be transfected with two vectors, the first vector containing an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed.

Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se. Such methods are shown, for instance, in references 12 and 13.

The DNA sequences which encode the A5B7 amino acid sequence may be obtained by methods well known in the art. For example, the A5B7 coding sequences may be obtained by genomic cloning, or cDNA cloning from the A5B7 hybridoma cell line. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for human immunoglobulin sequences may be obtained in any appropriate way. For example, DNA sequences coding for preferred human acceptor frameworks such as LAY, POM, KOL, REI, EU, TUR, TEI and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grated products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example, oligonucleotide directed synthesis as described by Jones et al (14) may be used. Also oligonucleotide directed mutagenesis of a pre-existing variable region as, for example, described by Verhoeyen et al (3) or Riechmann et al (4) may be used. Also enzymatic filling in of gapped oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (5) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the heavy and light chains of the antibody molecules. Bacterial e.g. E.coli, and other microbial systems may be used, in particular for expression of antibody fragments, e.g. Fv, Fab and Fab' fragments, and single chain antibody fragments e.g. single chain Fvs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

The present invention also includes therapeutic and diagnostic compositions containing the antibody molecule of the invention.

Such therapeutic and diagnostic compositions typically comprise an antibody molecule according to the invention in combination with a pharmaceutically acceptable excipient diluent or carrier, e.g. for in vivo use. Therapeutic and diagnostic uses typically comprise administering an effective amount of an antibody molecule according to the invention to a human subject.

Preferably the CDRs of the light chain correspond to the Kabat CDRs at CDR1 (positions 24-34) and CDR2 (positions 50-56) and to the structural loop residues (positions 91-96) or Kabat CDR residues (positions 89-97) in CDR3. In addition the light chain may have mouse residues at one or more of positions 1, 2 and/or 3, 46, 47, 49, 60, 70, 84, 85 and 87 and preferably has mouse residues at at least positions 46 and 47.

In addition to the CDRs, the HAM heavy chain preferably has mouse residues at positions 23 and/or 24 and 71 and/or 73. Additionally, the heavy chain may have mouse residues at one, some or all of positions 48 and/or 49, 69, 76 and/or 78, 80, 88 and/or 91 and 6. Preferably also, the CDRs of the heavy chain correspond to the Kabat CDR at CDR2 (positions 50-65), the structural loop residues at CDR3 (positions 95-100) and a composite of both the Kabat and structural loop residues at CDR1 (positions 24-35); for example, when the human variable region framework used is KOL. Alternatively, the CDRs of the heavy chain may comprise mouse residues at positions 26 to 35 for CDR1, positions 50 to 65 for CDR2 and positions 94 to 100 for CDR3; for example, when the human variable region framework used in EU. In addition EU has a particularly idiosyncratic J region between residues 103 to 113 and it may be useful to include the murine amino acids, or a consensus human J region or a suitable combination of both at residues 103 to 108 inclusive.

In a particularly preferred embodiment LAY human variable region frameworks are used for both the CDR-grafted heavy and light chains. In which case the light chain preferably comprises mouse A5B7 residues at positions 1, 2, 3, 4, 46 and 71, and especially also at positions 21, 47 and 73, of the variable region frameworks. Similarly, the heavy chain preferably comprises mouse A5B7 residues at positions 1, 24, 48, 49, 72, 73, 76 and 93, and especially also at positions 82b and 86, of the variable region frameworks. Also when the LAY human variable region frameworks are used, the variable regions preferably comprises A5B7 mouse CDRs at residues 24 to 34 (CDR1) 50 to 56 (CDR2) and 89 to 97 (CDR3) for the light chain and at residues 26 to 35 (CDR1), 50 to 65 (CDR3) and 95 to 102 (CDR3) for the heavy chain.

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering (15).

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is now described, by way of illustration only, in the following examples which refer to the accompanying diagrams Figures 1 - 16, in which:-

Figure 1 shows the DNA sequences encoding the unprocessed variable regions of the A5B7 MAb obtained by sequencing cDNA clones together with the predicted amino acid sequence;

Figure 2 is a schematic diagram of the construction by restriction and ligation of the chimeric heavy chain gene;

Figure 3 is a schematic diagram of the construction by site-directed mutagenesis, restriction and ligation of the chimeric light chain gene;

Figure 4 shows an ELISA analysis of COS-cell transfectant supernatants. The level of antigen-binding capacity

in the supernatant of COS-cell transfectants was analysed as described later. Dilution curves were plotted out against the optical density of the colour change;

Figure 5 shows plasmid diagrams for plasmids pBG7, pBG11, pBG14, pHMC19, pHMC20 and pHMC21;

Figure 6 shows plasmid diagrams for plasmids pHMC29, pHMC30 and pHMC31;

Figure 7 shows a SDS-PAGE gel of chimeric FAb' and chimeric DFM products under both reducing and non-reducing conditions;

Figure 8 shows the DNA and protein sequences for the A5B7 grafted light chain, gL-1 variable region;

Figure 9 shows similar sequences for the A5B7 grafted light chain gL-2, variable region;

Figure 10 shows similar sequences for the A5B7 grafted heavy chain gH1, variable region;

Figure 11 shows similar sequences for the A5B7 grafted heavy chain gH2, variable region.

Figure 12 shows plasmid diagrams for plasmids pMRR010, pMRR014, pAL43, pAL44, pAL45 and pAL46;

Figure 13 is graphs showing the results of direct CEA binding ELISAs on supernatants from transient expression of chimeric/grafted hybrids and a chimeric/chimeric standard;

Figure 14 shows similar graphs for grafted/grafted transfections as well as the chimeric/chimeric standard;

Figure 15 shows plasmid diagrams of plasmids pMRR020, pAL45, pAL46, pAL49 and pAL50 indicating the derivation of the latter two plasmids;

Figure 16 shows plasmid diagrams of various plasmids indicating the derivation of plasmids pHMC43, pHMC44, pAL53 and pAL54;

Figure 17 shows plasmid diagrams for pACtac, pMMR28 and pMRR45;

Figure 18 shows graphs of CEA binding ELISAs on E. coli supernatants containing A5B7 chimeric Fab', and

Figure 19 shows similar graphs for A5B7 grafted Fab'.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

Example 1

Molecular cloning and sequencing of the A5B7 heavy and light chain cDNAs

Polyadenylated RNA was isolated from the A5B7 hybridoma cell line using the guanidinium isothiocyanate/lithium chloride method (12). Double stranded cDNA was synthesised (16) and a cDNA library was constructed in plasmid pSP64 (17) vector using EcoRI linkers. Two screening probes were synthesised, complementary to mouse immunoglobulin heavy and light chain constant regions. The heavy chain probe was a 19 mer complementary to residues 115-133 in the CH1 domain of the mouse γ 1 sequence (18). The light chain probe was a 20 mer complementary to residues 4658-4677 of the genomic mouse CK sequence (19). The probes were radio-labelled at the 5' terminus with [γ ^{32}P] ATP using T4 polynucleotide kinase (Amersham International) and used to screen the cDNA library.

Clones which contained the complete leader, variable and constant regions of both the heavy and light chains were isolated and designated as pBG1 and pBG2. Nucleotide sequence analysis was carried out according to the chain termination procedure (20).

The 950 base pair EcoRI insert in pBG1 was fully sequenced. The EcoRI insert in pBG2 was shown to comprise approximately 1700 base pairs by agarose gel electrophoresis. The variable domain and the 5' region of the CH1 domain were sequenced, as was the 3' end of the clone to confirm the presence of the correct mouse γ 1 termination sequences. The DNA and predicted amino acid sequences for the unprocessed variable regions of pBG1 and pBG2 are shown in Figure 1.

With reference to Figure 1. Panel A shows the sequence coding for the V_L region and the predicted amino acid sequence. Panel B shows the sequence coding for the V_H region together with the predicted amino acid sequence. The putative sites of cleavage of the signal peptide are indicated by arrows. Examination of the derived amino acid sequence revealed considerable homology with other characterised immunoglobulin genes, and enabled the extent of the leader, variable and constant domains to be accurately determined. In addition MAb A5B7 was confirmed to be an IgG1 K antibody.

Example 2

Preparation and Testing of Chimeric Antibody Products

A. Construction of Chimeric Mouse-Human Heavy Chain Genes

The construction of vectors containing the human constant region isotype series, pRB18(IgG1), pRB26(IgG2), pRB20 (IgG3) and pRB21 (IgG4) is described in published International Patent Application WO 89/01783. The A5B7 V_H DNA sequence was isolated as a EcoRI-BanI fragment and was ligated to the following linking oligonucleotide to give an EcoRI-HindIII V_H fragment (Figure 2).

R1120 5′ GCACCACTCTCACCGTGAGCTC

R1121 GTGAGAGTGGCACTCGAGTCGA5′

This fragment was ligated to the human HindIII-BamHI containing fragments of the IgG1, 2, 3 & 4 genes cloned in pAT153 to give pBG3, 4, 5 & 6.

The chimeric heavy chain genes were isolated as ECORI-BamHI fragments from the pAT IgG plasmids and cloned into pEE6 vector (21) cut with EcoRI & Bc1I, to give plasmids pBG 7,8,9 & 10. The pEE6 plasmid contains the strong promoter/enhancer and transcriptional control element from the human cytomegalovirus immediate early gene (hCMV-IE) inserted into a unique HindIII site upstream of the EcoRI site and is described in detail in published International Patent Application WO 89/01036. In addition, an SV40 origin of replication is provided by the SV40 early promoter fragment which drives as selectable marker gene, a guanine phosphoribosyl transferase gene (gpt) inserted into a unique BamHI site. The plasmid also contains an ampicillin-resistance gene allowing selection and propagation in bacterial hosts.

B. Construction of the Chimeric Mouse-Human Light Chain Gene

A HindIII restriction enzyme site was introduced into the mouse light chain cDNA clone, pBG1 by site-directed mutagenesis (23) using the following oligonucleotide:-

5′ TTTGATTTCAAGCTTGGTGC 3′

Introduction of the HindIII site was verified by DNA sequencing. The A5B7 V_L sequence was isolated as an EcoRI/HindIII fragment. It was combined with a HindIII/EcoRI fragment comprising the human Ck constant region and ligated into the unique EcoRI site of pEE6. (Figure 3). The resulting plasmid pHMC19 also contained the neomycin-resistant gene (neo) under the control of the SV40 early promoter.

C. Transfections and ELISA Analysis of Antibody Production

The four chimeric heavy chain expression constructs described above were transfected together with chimeric light chain into COS-1 cells (24) for transient expression of chimeric products. The cells were left to incubate in DNA-DEAE dextran solution for six hours, then shocked for two minutes with 10% DMSO in HEPES-buffered saline. The cells were washed and incubated in medium containing 10% foetal calf serum for 72 hours.

Following incubation at 37°C for 72 hours the cell supernatants were analysed by ELISA for heavy and light chain production and binding of antigen.

The medium (500 µl per 10^5 cells) was removed for ELISA analysis.

To quantify assembled antibody production Microtitre plates were coated with 0.25 µg per well of sheep antibody reactive against human specific epitopes on the heavy or light chains. Supernatants or lysates from transfected COS cells were diluted 1:2 or 1:4 respectively in sample conjugate buffer containing 0.1M Tris-HC1 pH 7.0, 0.1M sodium chloride, 0.02% Tween 20 and 0.2% casein. 100 µl of each diluted sample were added to each well and incubated for 1 hour at room temperature with gentle agitation. Following washing six times with wash buffer (phosphate buffered saline containing 0.2% Tween 20, pH 7.2), 100 µl of 1:5000 dilution of standard horseradish peroxidase - conjugated

antibody reactive against human specific epitopes were added per well. The plates were incubated for 1 hour at room temperature, and then washed six times with wash buffer. 100 µl of substrate buffer containing 0.1 mg/ml tetramethylbenzidine (TMB), 0.1M sodium citrate, pH 6.0 and 0.005% H_2O_2 were added to each well to generate a colour change. The reaction was terminated after 2-3 minutes by adjusting the solution to pH 1.0 with 1.5M sulphuric acid. The optical density was determined at 450nm for each well by measurement in a Dynatech laboratories MR600 microplate reader. Standard curves were generated using known concentrations of the appropriate human immunoglobulins.

Antigen binding assays were performed in an analogous manner. Microtitre plates were coated with 0.25 µg per well of purified CEA. Following washing six times in wash buffer, samples from COS-cell transfections were added as previously, and the same subsequent procedures carried out, using goat anti-mouse or -human $F(ab')_2$ linked to HRP as the second antibody.

Assembly assays, which detect the presence of associated polypeptide chains, demonstrated the formation of multimers containing at least one heavy and one light chain when both genes were co-transfected. Antigen binding analysis (see above) demonstrated that the chimeric heavy and chimeric light chain co-transfections generated an antibody molecule capable of recognising antigen. The antigen binding ELISA data from one experiment are presented in Figure 4. These experiments demonstrate that chimerisation of the antibody molecule does not have a significant effect on its antigen recognition capability.

D. Immunoprecipitation of Antibody Molecules from Biosynthetically Labelled COS-Cell Transfectants

Following transfection, COS cells were allowed to recover for 24 hours in DMEM containing 10% foetal calf serum. The medium was then replaced with methionine-free DMEM, to which [^{35}S] methionine (NEN) had been added at 100 µCi/ml. The cells were metabolically labelled for 48hours. Analysis of the assembly and secretion of antibody molecules was performed by immunoprecipitation using anti-human $F(ab')_2$ bound to Protein A-Sepharose. Affinity-purified rabbit antibodies against human IgG $F(ab')_2$ were used for immunoprecipitations, following coupling to Protein A - Sepharose. Secreted antibodies were analysed on a SDS-10% PAGE system under reducing and non-reducing conditions. The gel was treated with an autoradiography enhancer, dried and exposed to Fuji RX film.

The antiserum immunoprecipitated proteins with an apparent molecular weight of 55K and 28K, corresponding to the heavy and light immunoglobulin chains respectively. A comparison of immunoprecipitations analysed by reducing and non-reducing SDS-PAGE indicated that the heavy and light chains were assembled as the correct tetrameric molecule.

Example 3

Preparation and Comparison of Chimeric Whole Antibody and Fab' Products

Stable cell lines expressing chimeric whole antibody and Fab' products were established and chimeric whole antibody, Fab', $F(ab')_2$, and synthetically cross-linked DFM (Di Fab' Maleimide) products were prepared and tested.

First of all, however, it was necessary to construct a DNA sequence coding for the chimeric Fab' and vectors for expression of this sequence.

A. Construction of Chimeric Mouse/Human Heavy Chain Gene and Vectors for Fab' Expression

The plasmid containing the A5B7 chimeric heavy chain, IgG4, (pBG10) was restricted with BstE11 and Bg111. The larger vector fragment containing the hCMV promoter and A5B7 V_H plus the 5' part of CH1 domain was isolated. The plasmid pJA115 (described in International Patent Application WO 89/01974) was restricted with BstE11 and Bg111. A fragment containing the 3' end of CH1 plus the IgG4 hinge containing a cys to ala change was isolated and ligated into the pBG10 vector.

The resulting vector, pBG14, contains the A5B7 Fd' heavy chain IgG4 (cys to ala).

The Assay procedures used in this and subsquent Examples were as follows:

Assembly ELISAs

The ELISA for measuring yields of whole antibodies used microwell plates coated with goat $F(ab')_2$ IgG Fc. Humanised IgG bound following incubation with culture supernatant samples was revealed with horseradish peroxidase (HRP) conjugated murine anti-human kappa chain antibody. Concentrations of chimeric or CDR-grafted whole antibody in samples were interpolated from a calibration curve generated from serial dilutions of purified chimeric A5B7 IgG1.

The ELISA for measuring yields of Fab's used microwell plates coated with murine anti-human IgG Fd. Following incubation with samples bound humanised Fab' was revealed as in the whole antibody assembly ELISA. Concentra-

tions of chimeric or CDR-grafted A5B7 Fab' in samples were interpolated from a calibration curve generated from serial dilutions of purified chimeric A5B7 Fab'.

CEA Binding Assays

The direct CEA binding ELISA used microwell plates coated with CEA. Following incubation with serial dilutions of culture supernatant samples bound IgG or fragments was revealed as for the assembly ELISA. Binding versus dilution curves were normalised against antibody concentration as determined by the assembly assays.

The competition RIA for anti-CEA activity involved competition of a series of ^{125}I-labelled murine or chimeric A5B7 IgG1 with humanised IgG or fragments thereof from culture supernatant samples for binding to CEA coated beads. Binding activity was determined by measuring bead associated radioactivity. The assay was calibrated by competition with standard preparations of chimeric or murine A5B7 IgG1 and plotting % bound cpm versus antibody concentration. Interpolated apparent A5B7 concentration of unknowns was normalised by dividing by the assembly assay result to give a specific activity. Finally specific activity was expressed as % relative potency by comparison to that obtained from a positive control chimeric A5B7 culture supernatant produced during the same experiment.

The relative potency of purified murine and chimeric A5B7 Fab' and F(ab')$_2$ fragments and murine IgG was investigated using the competition RIA. In addition, the direct binding ELISA was run in competition mode, by coincubation of the test specimen with murine A5B7 IgG1, for confirmation of relative potency.

Chimeric A5B7 Fab' was purified by ion-exchange chromatography on DEAE-sepharose followed by hydrophobic interaction chromatography on octyl-sepharose. Cross-linking was carried out by the standard one pot procedure using 1,6-bismaleimidohexane as cross-linker, with a 2.2 fold excess of Fab' to cross-linker at 0.9mg/ml. Due to the small scale of the experiment purification was carried out by HPLC gel filtration (GF-250XL). This yielded an A5B7 chimeric DFM (Di Fab' maleimide) product.

B. Development of CHO Cell Lines expressing Chimeric A5B7 IgG1 and Chimeric IgG4 Fab' delta cys

Two types of CHO cell line, amplifiable and non-amplifiable, were developed expressing chimeric A5B7 IgG1 whole antibody and chimeric A5B7 IgG4 FAb' delta cys. The chimeric A5B7 IgG1 whole antibody was used as a standard for assay development and for comparison of chimeric and grafted in biodistribution and therapy studies in tumour-bearing mice.

Non-amplifiable cell lines

Non-amplifiable cell lines for chimeric whole antibody and Fab' were first constructed. Although such cell lines are relatively low yielding, they are more readily and rapidly prepared and were used for rapid generation of material for development of procedures for purification and for assays. pHMC19 (see Example 2) is a plasmid containing the chimeric A5B7 light chain 3' to the hCMV promoter. This plasmid was transfected into CHO-K1 cells with selection for neomycin resistance and spot assays performed on 30 transfectants with anti-human kappa antibody to identify the best producing clones. Assays with the same antibody on representative cell lines identified a stable cell line, designated HCN1.37, secreting the chimeric A5B7 light chain with a specific production rate of 1-2 μg/ml/10^6 cells. The cell line was retransfected with plasmids pBG7 and pBG14, as described previously, these plasmids carrying the heavy chain genes for chimeric A5B7 IgG1 and chimeric IgG4 Fab' delta cys respectively, in the pEE6hCMV gpt vector (see Figure 5). Cell lines yielding approximately 16mg/l chimeric IgG1 (designated HCN1.37/gl.1 and g1.7) and approximately 5mg/l chimeric Fab' (designated HCN1.37/delta cys3) after purification were identified among these retransfectants by assembly and direct CEA binding assays. These cell lines were used to make test quantities of chimeric whole antibody and Fab'.

Amplifiable Cell Lines

Cell lines capable of amplification by the GS vector system and intended to give yields of chimeric A5B7 Fab' delta cys suitable for eventual manufacture were then constructed. The Cla1-EcoR1 fragment of pBG11 carrying the hCMV promoter and the chimeric A5B7 light chain gene was first cloned between the Cla1 and EcoR1 sites of pEE12 (22) to give plasmid pHMC20 (see Figure 5). Plasmid pHMC30 (see Figure 6) carrying the genes for both the chimeric light chain and chimeric IgG4 Fab' delta cys heavy chain was constructed by ligating the large Nae1-Fsp1 fragment of pHMC20 (carrying the light chain gene) to the EcoRV-Fsp1 fragment of pHMC28, a derivative of pBG14 in which the gpt gene has been removed from the BamHI site. pHMC30 is a double gene plasmid suitable for development of an amplifiable cell line, using the mouse myeloma cell line NSO as host cell line, expressing chimeric A5B7 Fab' since it contains the GS cDNA for selection of transfectants. Selection and amplification of transfectants in CHO cells requires

expression of the GS minigene rather than the cDNA. A double gene plasmid suitable for development of an amplifiable CHO cell line was constructed by ligating the MluI-FspI fragment of pHMC30, carrying both the chimeric light and heavy chain expression units, with the GS minigene containing fragment of pEE14 (25). The resulting plasmid, designated pHMC31 (see Figure 6), was transfected into CHO-K1 cells with selection on 25μM MSX. Specific production rates were determined on these transfectants, and seven chosen for amplification. The specific production rates for these seven transfectants before amplification were as follows (in units of $\mu g/10^6$ cells/24hrs): HC3.36, 1.3; HC3.21, 0.65; HC3.33, 0.65; HC5.19, 0.14; HC5.24, 3.4; HC5.33, 3.7; HC5.39, 0.35. Selection for cells potentially with high copy number of the inserted plasmid sequences was achieved by increasing the MSX concentration to between 100 and 1000 μM, screening for surviving cell lines and measuring specific production rates. Specific copy number estimates were not done.

C. Purification of Chimeric A5B7 IgG1

Chimeric IgG1 was purified by a modification of the procedure described for B72.3 by Colcher et al (26). CHO cell supernatants were concentrated by spiral cartridge ultrafiltration, then purified by affinity chromatography on protein A Sepharose, with elution at pH3. Reducing and non- reducing SDS-PAGE showed the purified antibody to be fully assembled, with a purity of > 95%.

D. Purification and Cross-linking of Chimeric A5B7 Fab'

Chimeric A5B7 Fab' was purified by ion-exchange chromotography on DEAE-Sepharose following by hydrophobic interaction chromotography on octyl-Sepharose. CHO cell culture supernatant containing A5B7 Fab' was concentrated ten fold by ultrafiltration and diluted to the original volume with 10mM tris pH 7.5 to reduce the conductivity to < 4 mS. This material was then applied to a column of DEAE-Sepharose fast flow pre-equilibrated with 10mM tris pH 7.5, and the flow through which contains the Fab' collected. The flow through from the DEAE-Sepharose column was then concentrated by ultrafiltration and made 2M in ammonium sulphate. Any precipitate was removed by centrifugation and the sample then applied to a column of octyl-Sepharose pre-equilibrated with 10mM tris pH 7.5 containing 2M ammonium sulphate. The Fab' bound to the column and was washed with equilibration buffer and eluted by decreasing the ammonium sulphate concentration to 1M. The elute was then dialysed into 100mM sodium acetate/citrate pH6 and concentrated by ultrafiltration.

The purified Fab' was cross-linked by firstly generating a free thiol at the hinge followed by cross-linking with 1,6-bismaleimidohexane. Partial reduction to generate a free thiol was achieved by incubation of the Fab' with 4.5mM β-mercaptoethylamine for 30 minutes at 37°C. The reducing agent was then removed by desalting on a column of Sephadex G-25 and the reduced Fab' immediately cross-linked by incubation with 1,6-bismaleimidohexane at a molar ratio of 1:2.2 bismaleimidohexane:Fab' with a Fab' concentration of 0.9 mg/ml. After overnight incubation at 37°C the cross-linked material was purified by HPLC gel filtration using a DuPont Zorbax GF-250XL column in 0.2M phosphate pH 7.0. The cross-linking yield was approximately 58%.

The purified Fab' and cross-linked di-Fab (DFM) were analysed by SDS-PAGE under both reducing and non-reducing conditions (Figure 7). The purified Fab' ran as expected with a molecular weight of approximately 50 KDa under non-reducing conditions which reduced to Fd' and light chains at 25KDa. The purified cross-linked di-Fab revealed the expected molecular weight for the non-reduced dimer of 100 KDa which reduced to cross-linked Fd' at approximately 50 KDa and light chain at approximately 25 KDa.

E. Antigen Binding Activity of Chimeric A5B7 IgG1, Fab' and DFM

The relative potencies of murine and chimeric IgGs and fragments were measured by the competition RIA. The results are given in Table 1. All the bivalent species - including the chimeric DFM - gave potency equivalent to that of murine A5B7 IgG. The murine Fab' rendered monovalent by alkylation, showed a tenfold reduced potency, as would be predicted by avidity considerations. The monovalent chimeric Fab', however, displayed an intermediate potency. Measurements of binding activity on the same samples using the direct binding ELISA in competition mode gave very similar results, with the chimeric Fab' again showing an intermediate potency. Since the chimeric Fab' was not alkylated its increased binding activity compared to the murine Fab' is likely to be due to some degree of antigen induced dimerisation.

Table 1

Relative potencies of various A5B7 constructs by competition RIA	
Construct	% Relative Potency
Chimeric IgG	100
Murine IgG	93 + 23
Chimeric DFM	104 + 28
Murine $F(ab)_2$	97 + 22
Chimeric Fab'	20 + 15
Murine Fab'	8 + 5

Example 4

Humanised, CDR-grafted versions of A5B7 were also prepared.

Construction and Expression of CDR-grated A5B7 Genes

Figure 1 shows the DNA and amino acid sequences of the V_L and V_H domains for A5B7. CDR-grafted V_H and V_L domains were designed substantially as described in International Patent Application No. PCT/GB90/02017 (publication No. WO91/09667). The amino acid sequence of A5B7 V_H shows considerable homology to the consensus sequence of the human V_H III subgroup, as defined by Kabat et al, (15), while the V_L sequence shows considerable homology to those of the human VII and III subgroups. The human framework sequences available within these subgroups are: LAY(V_H:v_L), POM(V_H:V_L), KOL(V_H) :REI(V_L), KOL(V_H): EU(V_L), TUR(V_H) ; REI(V_L), TUR(V_H):EU(V_L), TEI (V_H):REI(V_L), TEI(V_H):EU(V_L).

Of these LAY was chosen as the human framework because it has the highest homology to A5B7 and also the potential advantage of match V_H and V_L chains. CDR sequences and other residues potentially important for antigen binding were identified as described in International Patent Application No. PCT/GB90/02017, (Publication No. WO91/09967), and for each V region two constructs were assembled. The first constructs, gL1 and gH1, contain murine sequences in the CDRs and at other positions predicted to be important for antigen binding and at which human and A5B7 sequences differ. The gL1 light chain has murine CDRs at residues 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3) and additional murine residues within the frameworks at residues, 1, 2, 3, 4, 46 and 71. The gH1 heavy chain has murine CDRs at residues 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3) and additional murine residues within the frameworks at residues 1, 48, 49, 72, 73 and 76 and 93.

The second constructs, gL2 and gH2, are more conservative, containing the murine sequences present in the first constructs together with three extra murine residues for V_L and two for V_H. For gL2 these residues (positions 21, 47 and 73 on the Kabat numbering system) are potentially involved in packing of the domain. For gH2 the extra murine residues are at positions (82b and 86) where LAY has amino acids which are unusual for human V_H sequences and where A5B7 has residues of more common occurrence in human V_Hs.

Figures 8, 9, 10 and 11 show the DNA and amino acid sequences of gL1, gL2, gH1 and gH2 respectively. In these Figures single letter underlining in the amino acid sequences indicate a residue in the framework region which has been changed to the corresponding murine residue. Also in these Figures solid single line underlining of the amino acid sequences indicates the CDR residues. These DNA sequences were assembled from oligonucleotides (indicated by double line underlining in the respective Figure) by the PCR overlap-assembly procedure, using a Polymerase Chain Reaction (PCR) procedure, using oligonucleotides of approximately 80 bases alternating on the sense and antisense strands. The oligonucleotides overlapped by 20 bases, such that annealing led to the formation of partially double stranded molecules. The gaps were filled in by Taq polymerase and the double stranded material amplified by PCR using short oligonucleotides corresponding to the sequence of the 5' end of each strand as amplifiers. The amplified fragments were digested with appropriate restriction enzymes to expose the restriction sites for cloning.

Amplified fragments of the appropriate size were digested with HindIII and ApaI for V_H, and BstB1 and Sp11 for V_L to expose the cloning sites. The V_H fragments were cloned into the expression vector pMRR014 and the V_L fraqments into pMRR010. pMRR010 and pMRR014 are hCMV expression vectors designed to accept humanised V regions so as to be readily expressed transiently in CHO or COS cells and then readily to be reconstructed to give a single vector capable of stable expression and gene amplification in NSO cells. This gave plasmids pAL43 (for gL1), pAL44 (gL2), pAL45 (gH1) and pAL46 (gH2) as shown in Figure 12. These plasmids contain the full length CDR-grafted antibody genes (IgG1 for heavy chain, kappa for light chain) in a configuration suitable for expression in CHO cells. Clones containing the correct sequence were identified by DNA sequencing.

In order to assess the CEA binding activity of the grafted chains co-expression, experiments in a transient CHO cell system were first performed with chimeric partners. Thus pAL43 and pAL44 were co-transfected into the CHO L761 h cell line (27) together with the chimeric A5B7 heavy chain expression plasmid pBG7, while pAL45 and pAL46 were co-transfected with the light chain expression plasmid pBG11. Co-transfections of pBG7 and pHMC19 were also performed to provide a chimeric/chimeric standard against which to compare activity of the chimeric/grafted hybrids. Figure 13 shows the results of direct CEA binding ELISAs on the crude supernatants resulting from these transfections, and indicates that all the grafted/chimeric hybrids show binding activity similar to that of the chimeric/chimeric standard. Considerable variation was observed between the various hybrids in the yields of antibody. This variation was also very apparent when grafted/grafted co-transfections were performed. Indeed, the yields observed for gL1/gH1 and gL2/gH1 were too low to permit reliable estimates of CEA binding activity. Both the gL1/gH2 and gL2/gH2 combinations, however, bound CEA at approximately 60% as well as the double chimeric antibody in the direct binding assay (see Figure 14). Competition RIAs were also performed on the crude CHO cell supernatants for gL1/gH2 and gL2/gH2. The results, shown in Table 2, show that these grafted variants displayed approximately 42% and 47% respectively of the potency of the chimeric in these more stringent assays, with gL2/gH2 showing slightly greater potency than gL1/gH2.

Table 2

Estimates of Anti-CEA Activity in Transfected CHO Cell Supernatants for Grafted Variants of A5B7 by Competition RIA		
Undiluted		
	Specific activity	relative potency
Construct		
Chimeric A5B7	1.17	100±6
Grafted A5B7 gL1/gH2	0.49	42±4
Grafted A5B7 gL2/gH2	0.55	47±3

Example 5

Similarly CDR-grafted A5B7 Fab' genes were constructed and expressed.

A. Construction of CDR-grafted A5B7 Fab' Genes

pMRR020 is a pEE6 gpt expression plasmid with restriction sites such that coding sequences of the human IgG4 CH1 and delta cys (i.e. single cysteine variant) hinge domains (see Figure 15). A fragment containing these two domains was isolated by performing a PCR reaction on pMRR020 using oligonucleotide R1053 as the forward primer and R2371 as the back primer as shown below.

R1053 5′ GTCGACAGACTAACAGACTGTTCC 3′

R2371 5′ ATGATCAATGAATTCATCATGGGGCTGATGGGCACGGGGGACCATATTT GGACTC 3′

Use of the latter primer results also in the removal of the inconvenient ApaI site in the hinge coding region. The PCR reaction produced a fragment of 320bp, which was cloned into pAL45 and pAL46 to give pAL49 and pAL50 respectively (Figure 15) carrying the full length CDR-grafted Fd delta cys genes. The CH1 and hinge domains and the cloning junction regions were sequenced to confirm the absence of secondary mutations.

B. Development of CHO Cell Lines Expressing CDR-grafted A5B7 IgG1 Whole Antibody and CDR-grafted A5B7 IgG4 Fab' delta cys genes

To make stable cell lines expressing CDR-grafted IgG1 whole antibody and CDR-grafted Fab' the gL1 and gL2 genes were first isolated as Cla1-EcoR1 fragments from pAL43 and pAL44 and cloned into the vector pMRR017, a derivative of pEE14 with a useful poly-linker inserted at the BamHI site to give plasmids pHMC36 and pHMC37 respectively (see Figure 16). The gH1(IgG1), gH2(IgG1), gH1(Fd delta cys) and gH2(Fd delta cys) genes were isolated as Hind111-BamH1 fragments from pAL45, 46, 49 and 50 respectively and cloned along with an EcoR1-Hind111 fragment carrying the SV40 polyA and hCMV promoter between the EcoR1 and BamH1 sites of pHMC36 to give plasmids

pHMC43 (gL1-gH1), pHMC44(gL1-gH2), pAL53 (gL1-gH1Fd delta cys) and pAL54 (gL1-gH2Fd delta cys). These GS double gene expression plasmids (see Figure 16) were transfected into CHO-K1 cells to give CDR-grafted IgG1 whole antibody and CDR-grafted Fab' producing cell lines substantially as described in previous Examples.

Example 6

Production of A5B7 antibody fragments in E. coli

Chimeric and CDR-grafted A5B7 Fab' fragments (the gL1gH2 CDR-grafted variant was used) were also expressed in an E. coli secretion system, this being the preferred expression host for large scale production of antibody fragments.

For expression/secretion in E. coli the natural signal sequences of the A5B7 heavy and light chains were first replaced with the signal sequence of the E. coli outer membrane protein ompA (Movva et al, 28). A 92 base pair fragment encoding the ompA signal sequence and including the ompA translation initiation region was assembled from oligonucleotides and cloned into the phagemid vector pSK+ (from Stratagene Cloning Systems) between the XhoI and HindIII sites. The DNA and amino acid sequence of the 92 base pair fragment were as follows:

```
                                        metlyslysthralailealailealavalala
TCGAGTTCTAGATAACGAGGCGTAAAAAATGAAAAAGACAGCTATCGCGATTGCAGTGGCA
   CAAGATCTATTGCTCCGCATTTTTTACTTTTTCTGTCGATAGCGCTAACGTCACCGT

leualaglyphealathrvalalaglnala
CTGGCTGGTTTCGCTACCGTAGCGCA
GACCGACCAAAGCGATGGCATCGCGTTCGA
```

A clone shown by DNA sequencing to carry the above sequence in pSK+ was designated pSKompA.

For the chimeric A5B7 light chain a 650 base pair Sac1-EcoR1 fragment encoding most of V_L and all of C kappa was isolated from pHMC19. A precise fusion of the chimeric Light chain to the ompA signal sequence was made by ligating this Sac1-EcoR1 fragment into pSKompA digested with hind111 and EcoR1 together with a 54 base pair HindIII-SacI fragment assembled from oligonucleotides and comprising the DNA sequence encoding the 3' region of the ompA signal sequence and the 5' region of A5B7 V_L. The sequence of the 51 base pair fragment was as follows:

```
5'  AGCTCAAACTGTTCTCTCCCAGTCTCCAGCAATCCTGTCTGCATCTC      3'
3'      GTTTGACAAGAGAGGGTCAGAGGTCGTTAGGACAGACGTAGAGGTCC  5'
```

A clone containing the correct sequence was identified by DNA sequencing and designated pSKompA-cLc.

For the chimeric A5B7 heavy chain a 580 base pair Ava11-EcoR1 fragment encoding most of V_H and all of the CH1 and (delta cys) hinge domains was isolated from pBG14. A precise fusion of the chimeric heavy chain to the ompA signal sequence was made by ligating the Ava11-EcoR1 fragment into pSKompA digested with Hind111 and EcoR1 together with a 120 base pair fragment assembled from oligonucleotides and comprising the DNA sequence encoding the 3' end of the ompA signal sequence and the 5' region of V_H. The sequence of the 120 base pair fragment was as follows:

```
5' AGCTGAGGTGAAGCTTGTGGAGTCTGGAGGAGGGTTGGTACAGCCTGGGGGTTCTCTGA
3'     CTCCACTTCGAACACCTCAGACCTCCTCCGAACCATGTCGGACCCCCAAGAGACT

GACTCTCCTGTGCAACTTCTGGGTTCACCTTCACTGATTACTACATGAACTGG      3'
CTGAGAGGACACGTTGAAGACCCAAGTGGAAGTGACTAATGATGTACTTGACCCAGG 5'
```

A clone containing the correct sequence was identified by DNA sequencing and designated pSKompA-cFd.

For expression of the chimeric Fab' the ompA-cLc fusion was then removed from pSKompA-cLc on a Xho1-EcoR1 fraqment and cloned into the expression vector pACtac digested with SalI and partially with EcoR1 (see Figure 17). pACtac was constructed by replacing the Amp^r selectable marker and pUC18-derived replication functions of the expression plasmid, pTTQ9 (Amersham International) with the Cm^r selectable marker and replication functions of pACYC184 (Chang & Cohen, 1978, J. Bacteriol. 134: 1141-1156). (Partial EcoR1 digestion was required because pACtac contains a second EcoR1 site, in the Cm^r gene.) A plasmid with ompA-cLc inserted adjacent to the tac promoter

was identified by restriction mapping and DNA sequencing and designated pMRR024.

The ompA-cFd fragment was removed from pSKompA-cFd as a Xho1-Sma1 fragment and cloned into pSP73 (Promega Corporation) digested with Sall and Pvu11, to give a plasmid designated pMRR027. The ompA-cFd fusion was then removed from pMRR027 as an EcoR1 fragment, which was cloned into pMRR024 partially digested with EcoR1. A clone carrying ompA-cFd oriented for transcription from the tac promoter (along with ompA-cLc) was identified by restriction mapping and DNA sequencing and designated pMRR028.

pMRR028 was transformed into E. coli strain W3110 (ATCC strain 27325). Strain W3110 (pMRR028) was grown in a 1.5L fermenter in medium containing chloramphenicol to maintain selection for retention of the plasmid. At a culture O.D 600nm of 10 expression of the A5B7 Fab' genes from the tac promoter was induced by adding the inducer IPTG to a final concentration of 1mM. Direct CEA binding assays were performed on crude culture supernatant samples taken from this culture at time points 0, 3, 4, 5, 6, 7 and 8 hours after induction. The results of these assays, together with that of a chimeric A5B7 Fab' standard made and purified from mammalian cells are shown in Figure 18. They demonstrate the accumulation of active chimeric A5B7 Fab' in the E. coli culture medium. SDS-PAGE analysis on the same crude culture medium samples revealed proteins with mobility of the size expected for Fab' heavy and light chains. N-terminal protein sequencing on these proteins revealed the N-terminal amino acid sequences of mature A5B7 heavy and light chains, showing that the ompA signal sequence had been accurately cleaved from both. Estimates (by OD 280nM measurements and by SDS-PAGE) for the chimeric Fab' in the E. coli culture medium were performed: the results suggested yields in excess of 30mg/L after purification.

For the CDR-grafted A5B7 Fab' light chain a 620 base pair Hph1-EcoR1 fragment encoding most of V_L for the gL1 variant and all of C kappa was isolated from pAL43. A precise fusion of the grafted light chain to the ompA signal sequence was made by ligating the Hph-EcoR1 fragment into pSKompA digested with Hind111 and EcoR1 together with a 62 base pair fragment assembled from oligonucleotides and encoding the 3' end of the ompA signal sequence and the 5' end of the CDR-grafted V_L. The sequence of the synthetic fragment was as follows:

AGCTCAGACTGTACTCACTCAGAGTCCAAGTAGTCTCAGTGTAAGTGTAGGTGATAGGGTAA

GTCTGACATGAGTGAGTCTCAGGTTCATCAGAGTCACATTCACATCCACTATCCCAT

A clone carrying the correct sequence was identified by DNA sequencing and designated pMRR034.

For the CDR-grafted A5B7 Fab' heavy chain gene a 720 base pair Pvu11-EcoR1 fragment encoding most of V_H for the gH2 variant and all of CH1 and the (delta cys) hinge domains was isolated from pAL50. A precise fusion of this grafted H chain to the ompA signal sequence was made by ligating this Pvu11-EcoR1 fragment into pSKompA digested with Hind111 and EcoR1 together with a very short fragment assembled from oligonucleotides and encoding the 3' end of the ompA signal and the 5' end of the CDR-grafted V_H. The sequence of the short adaptor fragment was as follows:

5' AGCTGAGGTGCAG 3'

3' CTCCACGTC 5'

A clone containing the correct sequence was identified by DNA sequencing and designated pMRR037.

For expression of CDR-grafted Fab' the ompA-gL1 fusion was taken from pMRR034 as a XhoI-EcoR1 fragment and cloned into pACtac digested with Sall and partially with EcoRI. A clone carrying ompA-gL1 adjacent to the tac promoter was identified by restriction mapping and DNA sequencing and designated pmRR038. The ompA-gH2 fusion was then taken from pMRR037 as a XhoI-Sma1 fragment and cloned into pSP73 doubly digested with Pvu11 and EcoR1 to give a plasmid designated pMRR041. The ompA-gH2 fusion was then removed from pMRR041 as an EcoR1 fragment and cloned into pMRR038 partially digested with EcoR1. A clone carrying the ompA-gH2 fusion oriented for transcription from the tac promoter (along with ompA-gL1) was identified by restriction mapping and DNA sequencing and designated pMRR045.

pMRR045 was transformed into E. coli strain W3110 and the W3110 (pMRR045) strain resulting was grown in a 1.5L fermenter. Expression of the CDR-grafted Fab' genes was induced as described above for the chimeric Fab' genes. Crude culture supernatant samples from this culture at time points 1, 4, 5, 6, 11 and 22 hours after induction were used in direct CEA binding assays and yield estimates. The results of the CEA binding assays are given in Figure 19 and show the accumulation of material active in antigen binding. SDS-PAGE analysis of these supernatant samples demonstrated the presence of proteins of the size expected, and suggested yields in excess of 30mg/L.

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Claims

1. An antibody molecule having specificity for carcinoembryonic antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain framework residues and heavy chain antigen-binding residues from the mouse monoclonal antibody A5B7, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 23 and/or 24, 26 to 35, 50 to 65, 71 and/or 73 and 95 to 100 are A5B7 residues.

2. The antibody molecule of claim 1, wherein residue 24 and additionally residue 25 in the heavy chain are A5B7 residues.

3. The antibody molecule of claim 1, wherein residue 94 in the heavy chain is additionally an A5B7 residue.

4. The antibody molecule of claim 1, wherein residues 101 and 102 are additionally A5B7 residues.

5. The antibody molecule of any one of claims 1 to 4, wherein residues 6, 48, 49, 69, 76, 78, 80, 88 and 91 are additionally A5B7 residues.

6. The antibody molecule of any one of claims 1 to 4, wherein the heavy chain framework residues are LAY residues.

7. The antibody molecule of claim 6, wherein residues 1, 24, 48, 49, 72, 73, 76 and 93 in the heavy chain are A5B7 residues.

8. The antibody molecule of claim 7, wherein residues 82b and 86 in the heavy chain are additionally A5B7 residues.

9. An antibody molecule having specificity for carcinoembryonic antigen and comprising a composite light chain and a complementary heavy chain, said composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and light chain antigen-binding residues from the mouse monoclonal

antibody A5B7, wherein, according to the Kabat numbering system, in said composite light chain, at least residues 24 to 34, 46, 47, 50 to 56 and 91 to 96 are A5B7 residues.

10. The antibody molecule of any one of claims 1 to 8, wherein the complementary light chain is a composite light chain having a variable domain comprising human acceptor antibody light chain framework residues and light chain antigen-binding residues from the mouse monoclonal antibody A5B7, wherein, according to the Kabat numbering system, in said composite light chain, at least residues 24 to 34, 46, 47, 50 to 56 and 91 to 96 are A5B7 residues.

11. The antibody molecule of claim 9 or claim 10, wherein residues 89 to 91 and 97 in the light chain are additionally A5B7 residues.

12. The antibody molecule of any one of claims 9 to 11, wherein residues 1 to 3, 49, 60, 70, 84, 85 and 87 in the light chain are additionally A5B7 residues.

13. The antibody molecule of any one of claims 9 to 12, wherein the light chain framework residues are LAY residues.

14. The antibody molecule of claim 13, wherein residues 1 to 4, 21, 71 and 73 in the light chain are additionally A5B7 residues.

15. The antibody molecule of any one of claims 1 to 14 when produced by recombinant DNA technology.

16. The antibody molecule of any one of claims 1 to 15, which comprises a complete antibody molecule, or a Fab, Fab', $(Fab')_2$ or Fv fragment or a single chain antibody fragment.

17. The antibody molecule of any one of claims 1 to 16, having an effector or reporter molecule attached thereto.

18. A therapeutic or diagnostic composition comprising an antibody molecule of any one of claims 1 to 17 in combination with a pharmaceutically acceptable excipient, diluent or carrier.

Patentansprüche

1. Antikörpermolekül mit einer Spezifität für carcinoembryonisches Antigen, welches Antikörpermolekül eine zusammengesetzte schwere Kette und eine komplementäre leichte Kette aufweist, welche zusammengesetzte schwere Kette einen variablen Bereich besitzt, der Gerüstreste einer schweren Kette eines menschlichen Akzeptor-Antikörpers und Antigenbindende Reste einer schweren Kette des monoklonalen Mausantikörpers A5B7 aufweist, wobei gemäß dem Kabat-Numerierungssystem in der zusammengesetzten schweren Kette mindestens die Reste 23 und/oder 24, 26 bis 35, 50 bis 65, 71 und/oder 73 und 95 bis 100 A5B7-Reste sind.

2. Antikörpermolekül nach Anspruch 1, wobei der Rest 24 und zusätzlich der Rest 25 in der schweren Kette A5B7-Reste sind.

3. Antikörpermolekül nach Anspruch 1, wobei der Rest 94 in der schweren Kette zusätzlich ein A5B7-Rest ist.

4. Antikörpermolekül nach Anspruch 1, wobei die Reste 101 und 102 zusätzlich A5B7-Reste sind.

5. Antikörpermolekül nach einem der Ansprüche 1 bis 4, wobei die Reste 6, 48, 49, 69, 76, 78, 80, 88 und 91 zusätzlich A5B7-Reste sind.

6. Antikörpermolekül nach einem der Ansprüche 1 bis 4, wobei die Gerüstreste der schweren Kette LAY-Reste sind.

7. Antikörpermolekül nach Anspruch 6, wobei die Reste 1, 24, 48, 49, 72, 73, 76 und 93 in der schweren Kette A5B7-Reste sind.

8. Antikörpermolekül nach Anspruch 7, wobei die Reste 82b und 86 in der schweren Kette zusätzlich A5B7-Reste sind.

9. Antikörpermolekül mit einer Spezifität für carcinoembryonisches Antigen, welches Antikörpermolekül eine zusammengesetzte leichte Kette und eine komplementäre schwere Kette aufweist, welche zusammengesetzte leichte Kette einen variablen Bereich besitzt, der Gerüstreste einer leichten Kette eines menschlichen Akzeptor-Antikörpers und Antigenbindende Reste einer leichten Kette des monoklonalen Mausantikörpers A5B7 aufweist, wobei gemäß dem Kabat-Numerierungssystem in der zusammengesetzten leichten Kette mindestens die Reste 24 bis 34, 46, 47, 50 bis 56 und 91 bis 96 A5B7-Reste sind.

10. Antikörpermolekül nach einem der Ansprüche 1 bis 8, wobei die komplementäre leichte Kette eine zusammengesetzte leichte Kette ist, mit einem variablen Bereich, der Gerüstreste einer leichten Kette eines menschlichen Akzeptor-Antikörpers und Antigen-bindende Reste einer leichten Kette des monoklonalen Mausantikörpers A5B7 aufweist, wobei gemäß dem Kabat-Numerierungssystem in der zusammengesetzten leichten Kette mindestens die Reste 24 bis 34, 46, 47, 50 bis 56 und 91 bis 96 A5B7-Reste sind.

11. Antikörpermolekül nach Anspruch 9 oder 10, wobei die Reste 89 bis 91 und 97 in der leichten Kette zusätzlich A5B7-Reste sind.

12. Antikörpermolekül nach einem der Ansprüche 9 bis 11, wobei die Reste 1 bis 3, 49, 60, 70, 84, 85 und 87 in der leichten Kette zusätzlich A5B7-Reste sind.

13. Antikörpermolekül nach einem der Ansprüche 9 bis 12, wobei die Gerüstreste der leichten Kette LAY-Reste sind.

14. Antikörpermolekül nach Anspruch 13, wobei die Reste 1 bis 4, 21, 71 und 73 in der leichten Kette zusätzlich A5B7-Reste sind.

15. Antikörpermolekül nach einem der Ansprüche 1 bis 14, hergestellt mittels rekombinanter DNA-Technik.

16. Antikörpermolekül nach einem der Ansprüche 1 bis 15, welches ein vollständiges Antikörpermolekül oder ein Fab-, Fab'-, $(Fab)_2$- oder Fv-Fragment oder ein einkettiges Antikörperfragment umfaßt.

17. Antikörpermolekül nach einem der Ansprüche 1 bis 16, an welchem ein Effektor- oder Reporter-Molekül angebracht ist.

18. Therapeutische oder diagnostische Zusammensetzung, welche ein Antikörpermolekül nach einem der Ansprüche 1 bis 17 in Kombination mit einem pharmazeutisch akzeptablen Exzipienten, Verdünnungsmittel oder Träger enthält.

Revendications

1. Molécule d'anticorps ayant une spécificité pour l'antigène carcino-embryonnaire et comprenant une chaîne composite lourde et une chaîne complémentaire légère, ladite chaîne composite lourde ayant un domaine variable comprenant des résidus de chaîne lourde de cadre de lecture d'anticorps d'accepteur humain et des résidus de chaîne lourde se liant à un antigène provenant de l'anticorps monoclonal de souris A5B7, dans laquelle, selon le système de numérotation de Kabat, dans ladite chaîne composite lourde au moins les résidus 23 et/ou 24, 26 à 35, 50 à 65, 71 et/ou 73 et 95 à 100 sont des résidus de A5B7.

2. Molécule d'anticorps selon la revendication 1, dans laquelle le résidu 24 et de plus le résidu 25 dans la chaîne lourde sont des résidus de A5B7.

3. Molécule d'anticorps selon la revendication 1, dans laquelle le résidu 94 dans la chaîne lourde est de plus un résidu de A5B7.

4. Molécule d'anticorps selon la revendication 1, dans laquelle les résidus 101 et 102 sont de plus des résidus de A5B7.

5. Molécule d'anticorps selon l'une quelconque des revendications 1 à 4, dans laquelle les résidus 6, 48, 49, 76, 78, 80, 88 et 91 sont de plus des résidus de A5B7.

6. Molécule d'anticorps selon l'une quelconque des revendications 1 à 4, dans laquelle les résidus de chaîne lourde de cadre de lecture sont des résidus de LAY.

7. Molécule d'anticorps selon la revendication 6, dans laquelle les résidus 1, 24, 48, 49, 72, 73, 76 et 93 dans la chaîne lourde sont des résidus de A5B7.

8. Molécule d'anticorps selon la revendication 7, dans laquelle les résidus 82b et 86 dans la chaîne lourde sont de plus des résidus de A5B7.

9. Molécule d'anticorps ayant une spécificité pour l'antigène carcino-embryonnaire et comprenant une chaîne composite légère et une chaîne complémentaire lourde, ladite chaîne composite légère ayant un domaine variable comprenant des résidus de chaîne lourde de cadre de lecture d'anticorps d'accepteur humain et des résidus de chaîne légère se liant à un antigène provenant de l'anticorps monoclonal de souris A5B7, dans laquelle, selon le système de numérotation de Kabat, dans ladite chaîne composite légère, au moins les résidus 24 à 34, 46, 47, 50 à 56 et 91 à 96 sont des résidus de A5B7.

10. Molécule d'anticorps selon l'une quelconque des revendications 1 à 8, dans laquelle la chaîne légère complémentaire est une chaîne composite légère ayant un domaine variable comprenant des résidus de chaîne légère de cadre de lecture d'anticorps d'accepteur humain et des résidus de chaîne légère se liant à un antigène provenant de l'anticorps monoclonal de souris A5B7, dans laquelle, selon le système de numérotation de Kabat, dans ladite chaîne composite légère, au moins les résidus 24 à 34, 46, 47, 50 à 56 et 91 à 96 sont des résidus de A5B7.

11. Molécule d'anticorps selon la revendication 9 ou la revendication 10, dans laquelle les résidus 89 à 91 et 97 dans la chaîne légère sont de plus des résidus de A5B7.

12. Molécule d'anticorps selon l'une quelconque des revendications 9 à 11, dans laquelle les résidus 1 à 3, 49, 60, 70, 84, 85 et 87 dans la chaîne légère sont de plus des résidus de A5B7.

13. Molécule d'anticorps selon l'une quelconque des revendications 9 à 12, dans laquelle les résidus de chaîne légère de cadre de lecture sont des résidus de LAY.

14. Molécule d'anticorps selon la revendication 13, dans laquelle les résidus 1 à 4, 21, 71 et 73 dans la chaîne légère sont de plus des résidus de A5B7.

15. Molécule d'anticorps selon l'une quelconque des revendications 1 à 14 produite par une technique d'ADN recombinant.

16. Molécule d'anticorps selon l'une quelconque des revendications 1 à 15, qui comprend une molécule complète d'anticorps, ou un fragment Fab, Fab' $(Fab')_2$ ou Fv, ou un fragment d'anticorps à chaîne simple.

17. Molécule d'anticorps selon l'une quelconque des revendications 1 à 16, ayant une molécule effecteur ou rapporteur attachée à celle-ci.

18. Composition destinée à une thérapie ou à un diagnostic comprenant une molécule d'anticorps selon l'une quelconque des revendications 1 à 17 en combinaison avec un excipient, diluant ou véhicule pharmaceutiquement acceptable.

Fig. 1

A5B7: light chain sequence

DNA and protein sequence of the VI domain

```
ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTCC
 M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S  V  I  M  S

AGAGGACAAACTGTTCTCTCCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAG
 R  G  Q  T  V  L  S  Q  S  P  A  I  L  S  A  S  P  G  E  K

GTCACAATGACTTGCAGGGCCAGCTCAAGTGTAACTTACATTCACTGGTACCAGCAGAAG
 V  T  M  T  C  R  A  S  S  S  V  T  Y  I  H  W  Y  Q  Q  K

CCAGGATCCTCCCCCAAATCCTGGATTTATGCCACATCCAACCTGGCTTCTGGAGTCCCT
 P  G  S  S  P  K  S  W  I  Y  A  T  S  N  L  A  S  G  V  P

GCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGAGTGGAG
 A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I  S  R  V  E

GCTGAAGATGCTGCCACTTATTACTGCCAACATTGGAGTAGTAAACCACCGACGTTCGG
 A  E  D  A  A  T  Y  Y  C  Q  H  W  S  S  K  P  P  T  F  G

GGAGGCACCAAGCTGGAAATCAAACGG
 G  G  T  K  L  E  I  K  R
```

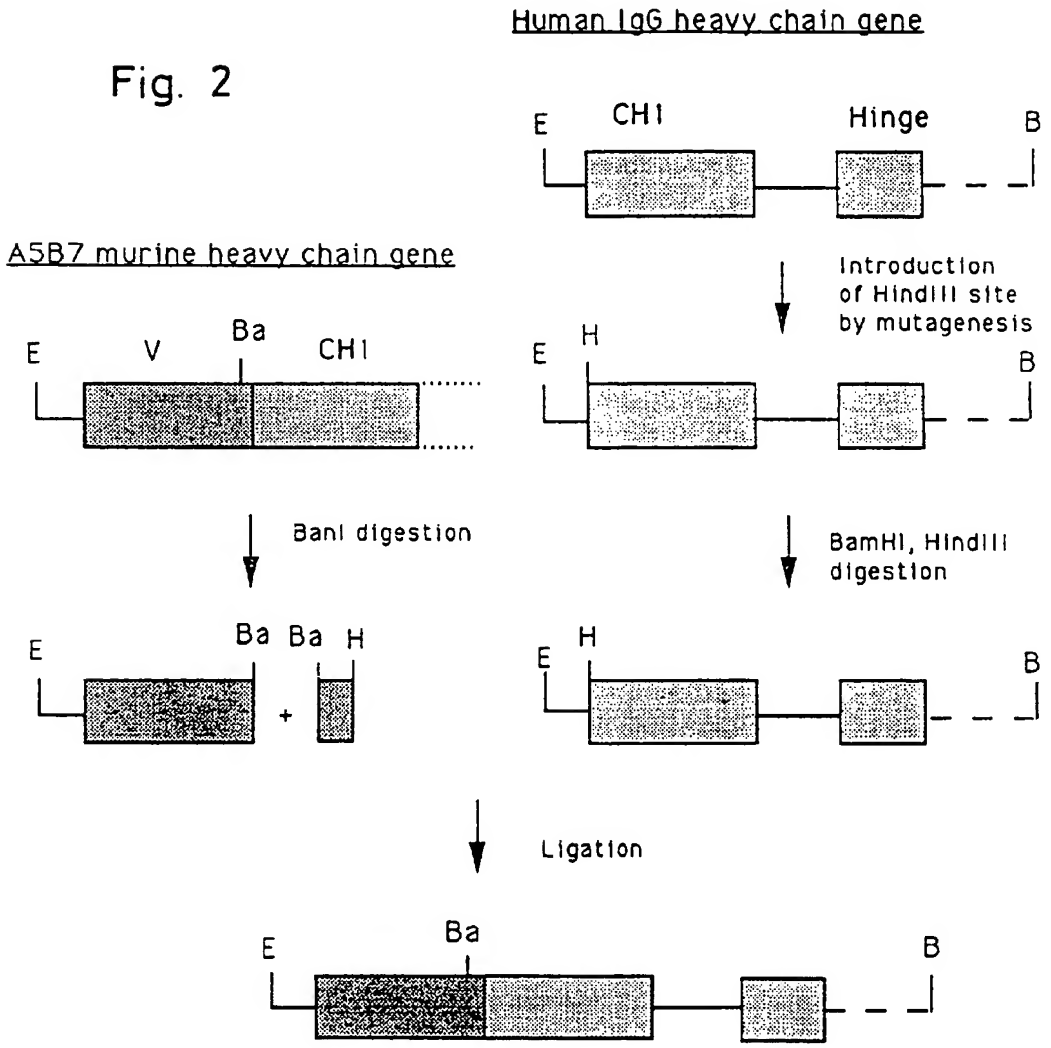
A5B7: heavy chain sequence

DNA and protein sequence of the Vh domain

```
ATGAAGTTGTGGCTGAACTGGATTTTCCTTGTAACACTTTTAAATGGTATCCAGTGTGAG
 M  K  L  W  L  N  W  I  F  L  V  T  L  L  N  G  I  Q  C  E

GTGAAGCTGGTGGAGTCTGGAGGAGGCTTGGTACAGCCTGGGGGTTCTCTGAGACTCTCC
 V  K  L  V  E  S  G  G  G  L  V  Q  P  G  G  S  L  R  L  S

TGTGCAACTTCTGGGTTCACCTTCACTGATTACTACATGAACTGGGTCCGCCAGCCTCCA
 C  A  T  S  G  F  T  F  T  D  Y  Y  M  N  W  V  R  Q  P  P

GGAAAGGCACTTGAGTGGTTGGGTTTTATTGGAAACAAAGCTAATGGTTACACAACAGAG
 G  K  A  L  E  W  L  G  F  I  G  N  K  A  N  G  Y  T  T  E

TACAGTGCATCTGTGAAGGGTCGGTTCACCATCTCCAGAGATAAATCCCAAAGCATCCTC
 Y  S  A  S  V  K  G  R  F  T  I  S  R  D  K  S  Q  S  I  L

TATCTTCAAATGAACACCCTGAGAGCTGAGGACAGTGCCACTTATTACTGTACAAGAGAT
 Y  L  Q  M  N  T  L  R  A  E  D  S  A  T  Y  Y  C  T  R  D

AGGGGGCTACGGTTCTACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
 R  G  L  R  F  Y  F  D  Y  W  G  Q  G  T  T  L  T  V  S  S
```

Construction of A5B7 chimeric heavy chain gene.

Fig. 2



E = EcoRI
B = BamHI
H = HindIII
Ba = BanI

Construction of A5B7 chimeric light chain gene.

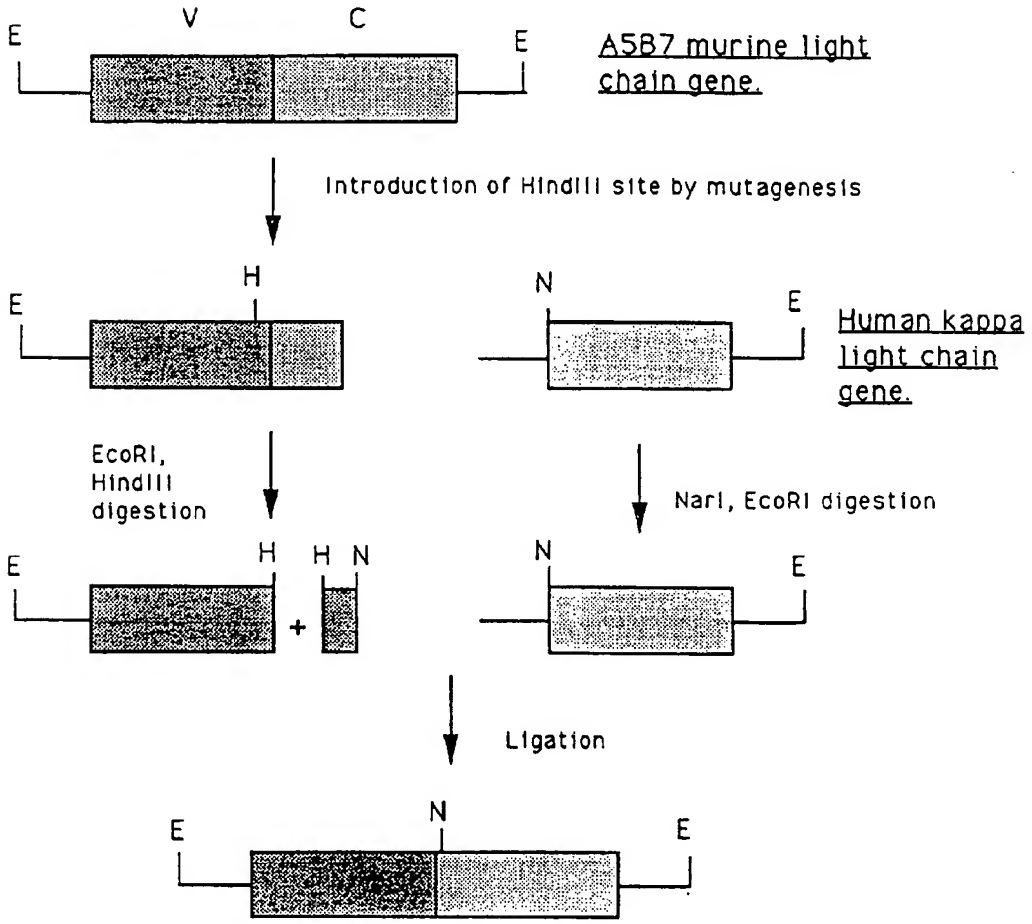
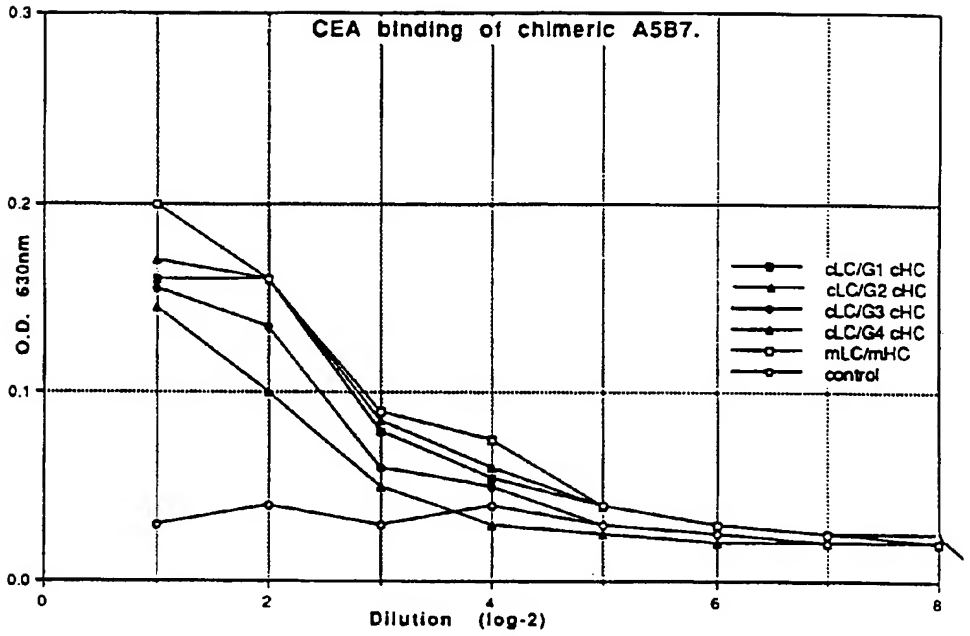


Fig. 3

E = EcoRI
H = HindIII
N = NarI

Fig. 4



cHC = chimeric heavy chain.

cLC = chimeric light chain.

mHC = mouse heavy chain.

mLC = mouse light chain.

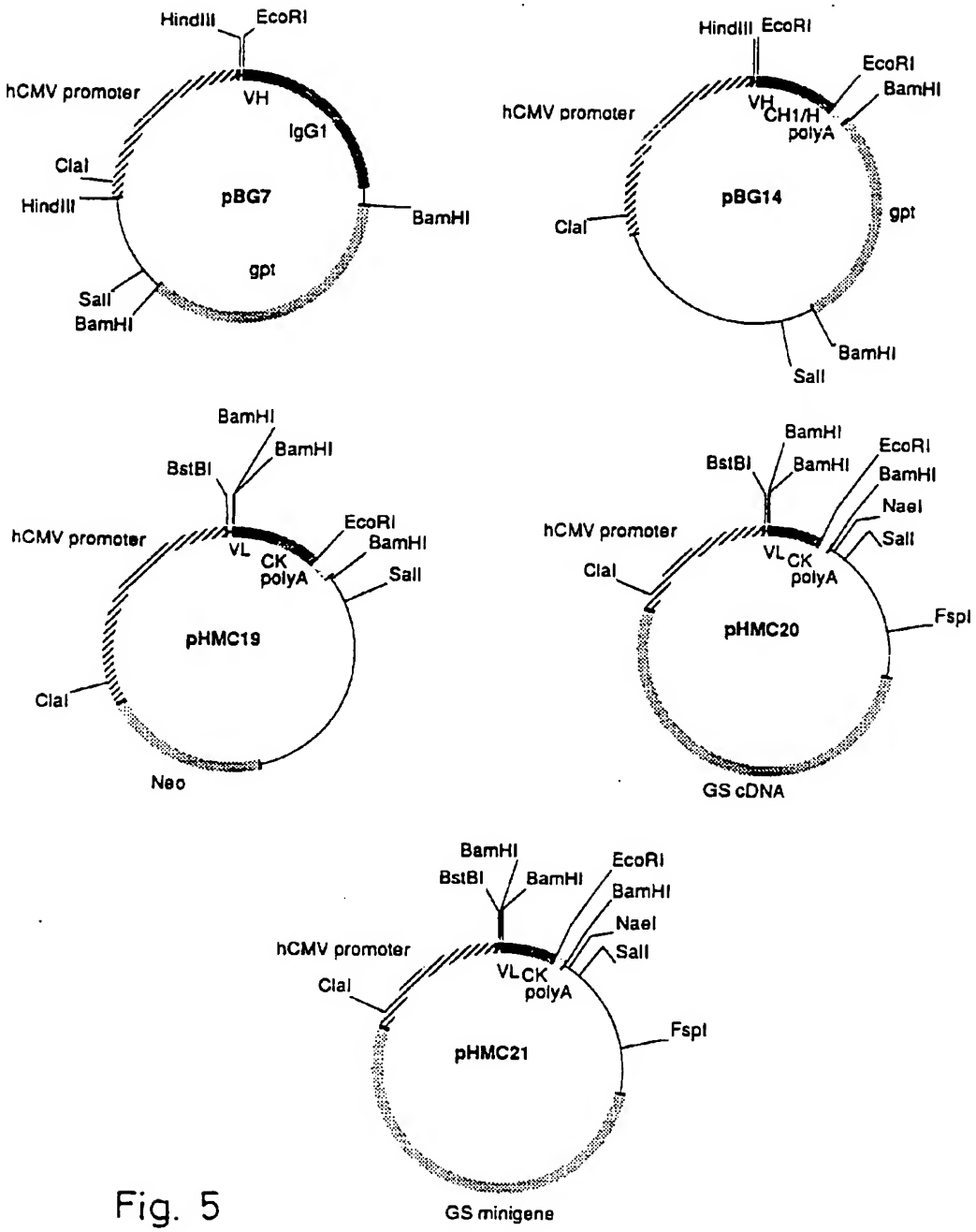
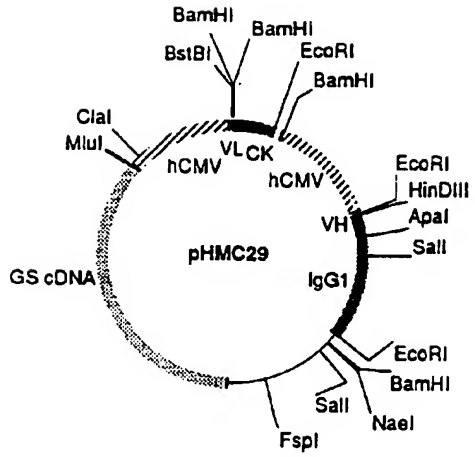


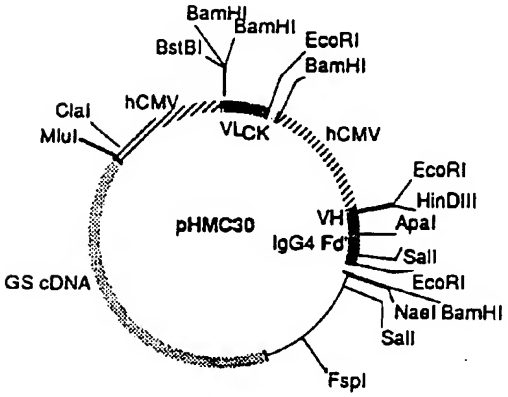
Fig. 5

VECTORS FOR cA5B7 EXPRESSION

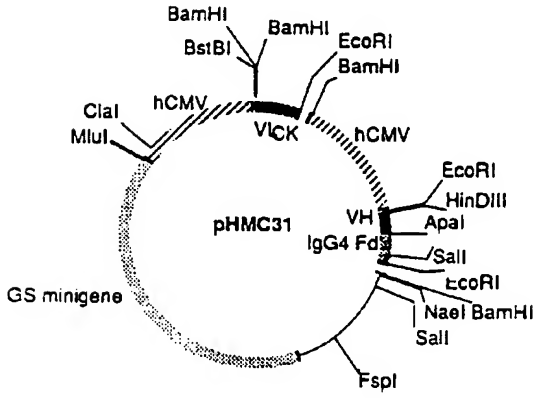
Fig. 6



GS vector for cL-cIgG1 expression in NS0 cells



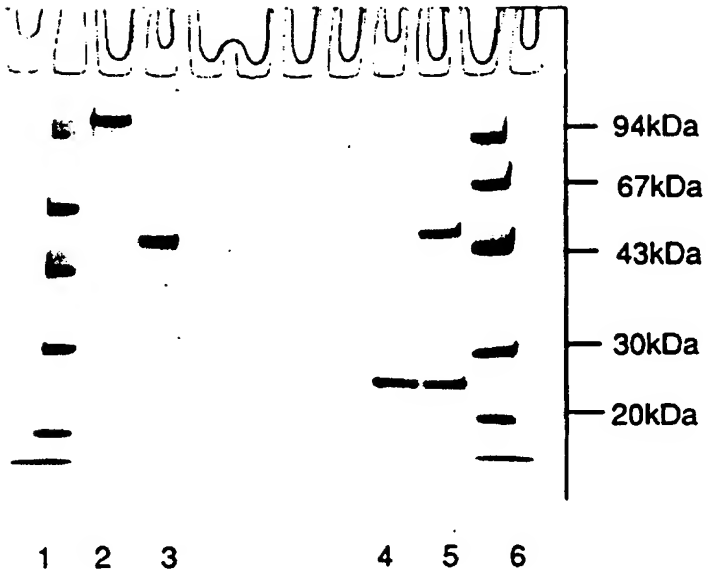
GS vector for cL-cIgG4Fd' expression in NS0 cells



GS vector for cL-cIgG4Fd' expression in CHO cells

GS VECTORS FOR cA5B7 IgG1 AND cA5B7 Fab'

Purification and cross-linking of A5B7 cFab'



1. Molecular weight markers, non-reduced
2. A5B7 cDFM, non-reduced
3. A5B7 cFab', non-reduced
4. A5B7 cFab', reduced
5. A5B7 cDFM, reduced

10-20% acrylamide gradient gel, Coomassie blue stained

Fig. 7

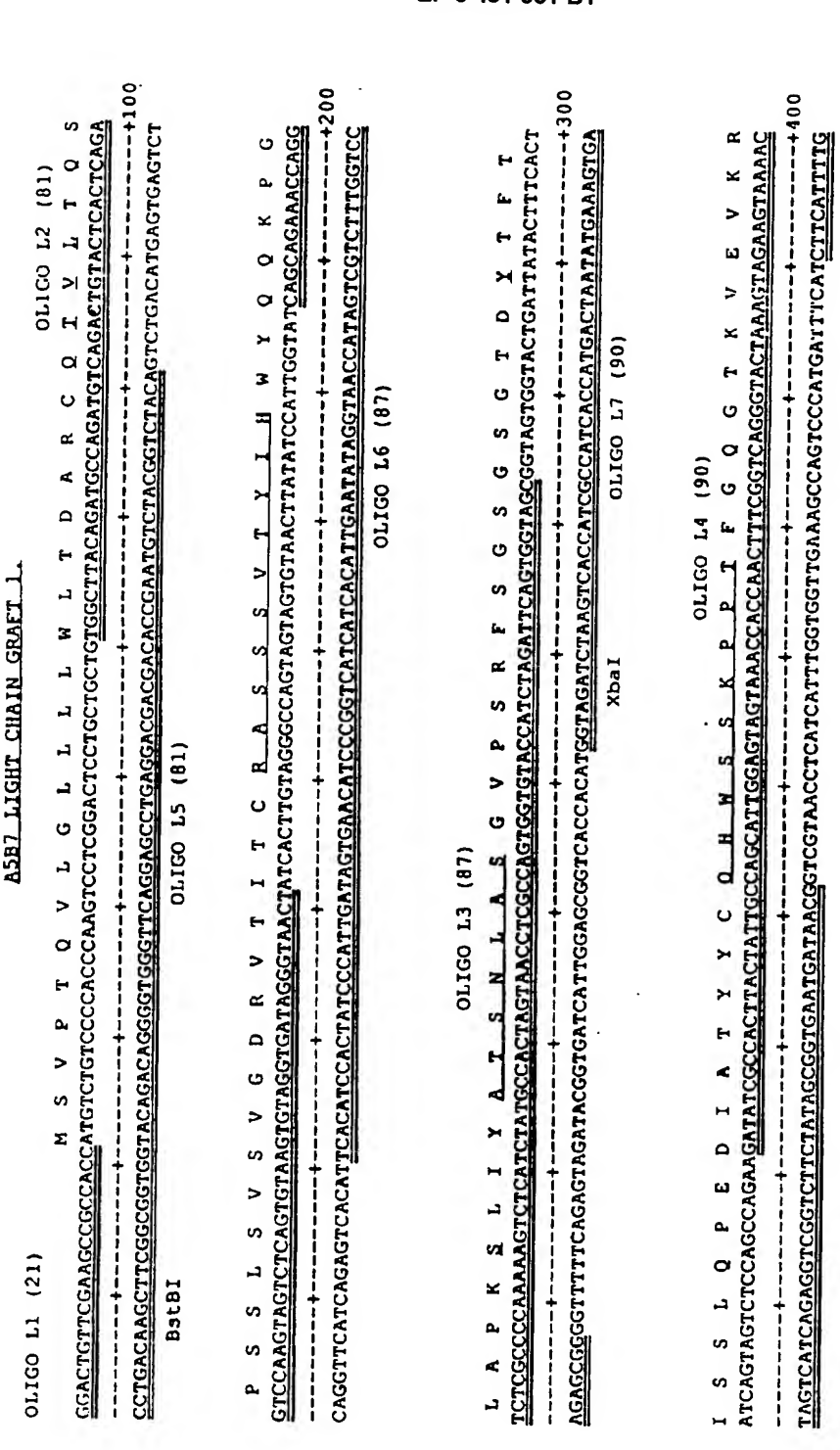


Fig. 8

DNA AND PROTEIN SEQUENCE FOR
gL1-A5B7 VARIABLE REGION

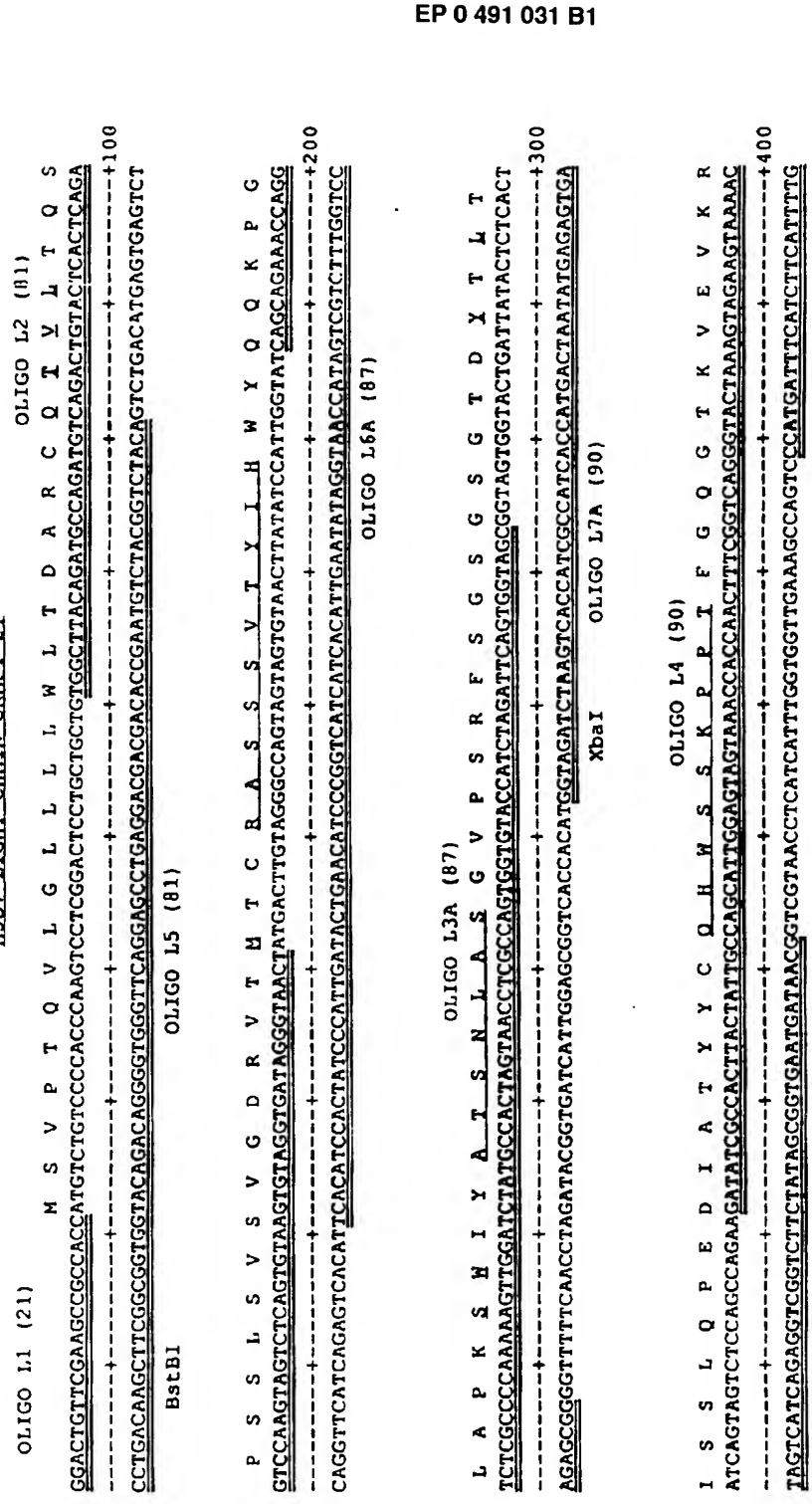


Fig. 9

DNA AND PROTEIN SEQUENCE FOR
gL2-A5B7 VARIABLE REGION

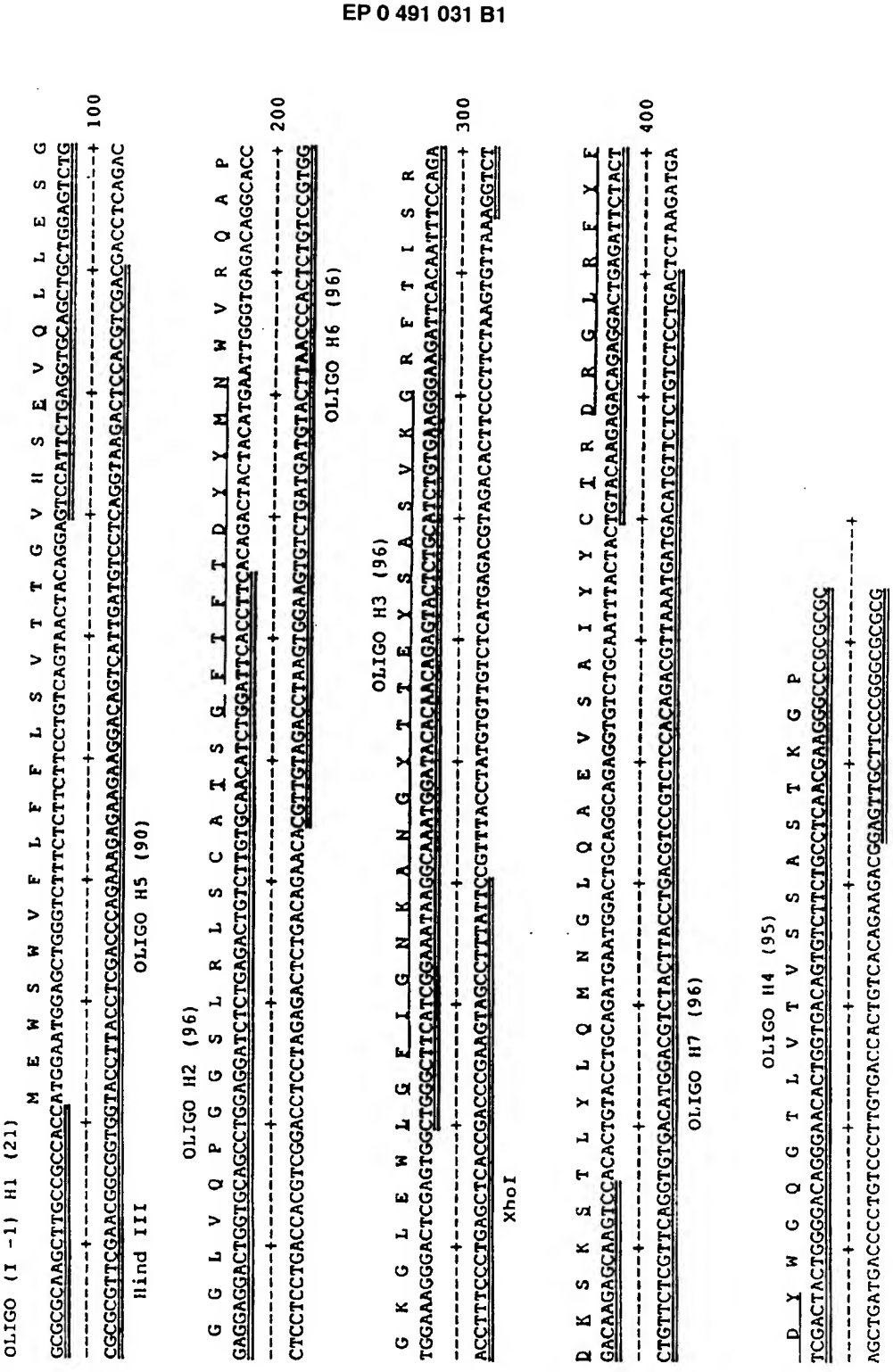


Fig. 10

DNA AND PROTEIN SEQUENCE FOR
gH1-A5B7 VARIABLE REGION

Fig. 11

DNA AND PROTEIN SEQUENCE FOR
gH2-A5B7 VARIABLE REGION

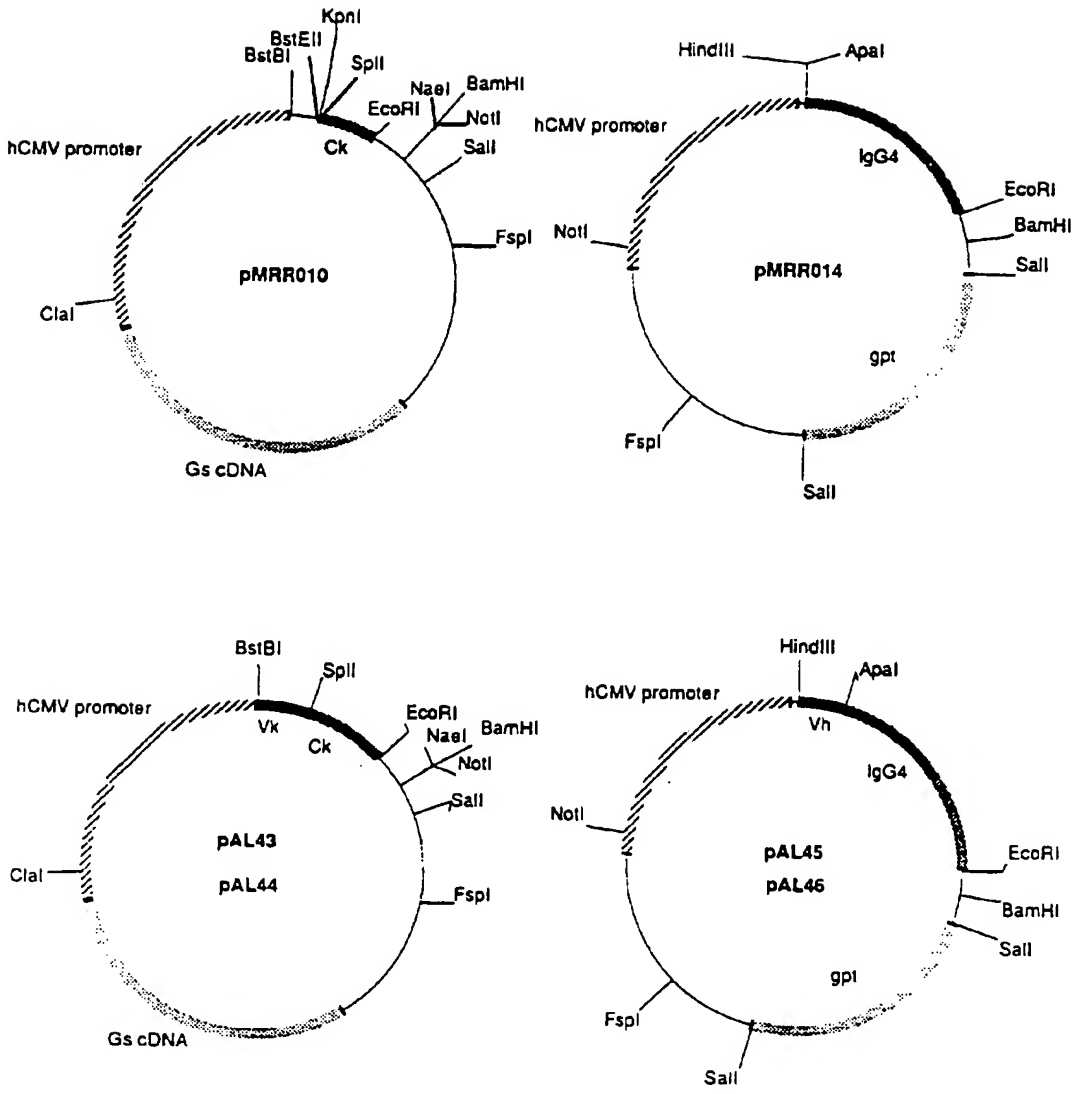


Fig. 12

VECTORS FOR gA5B7 EXPRESSION

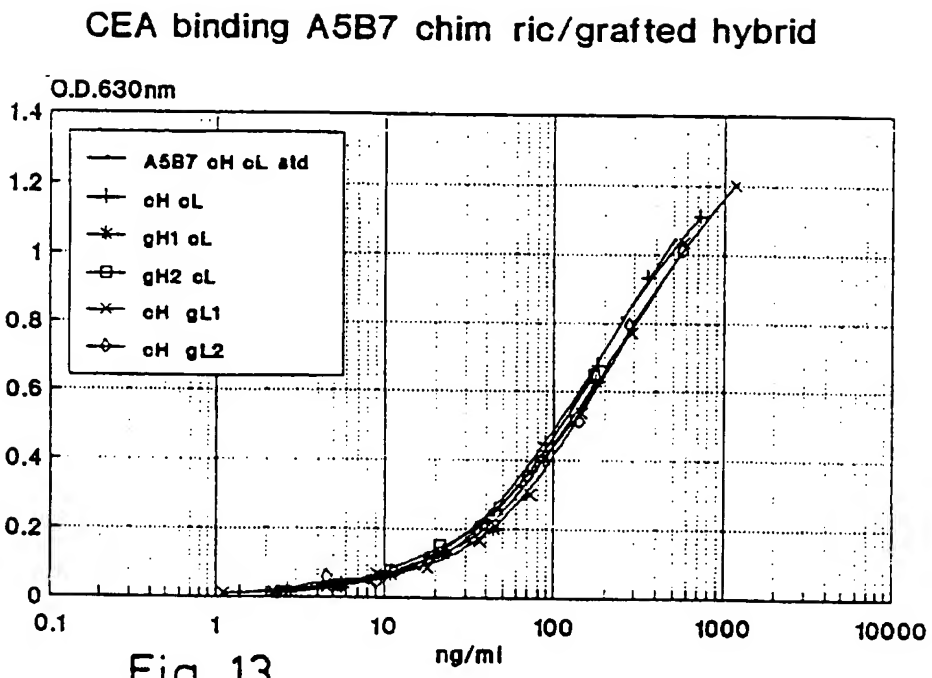

CEA binding A5B7 chim ric/grafted hybrid
O.D.630nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A5B7 cH cL std
cH cL
gH1 cL
gH2 cL
cH gL1
cH gL2
0.1
1
10
100
1000
10000
ng/ml

Fig. 13

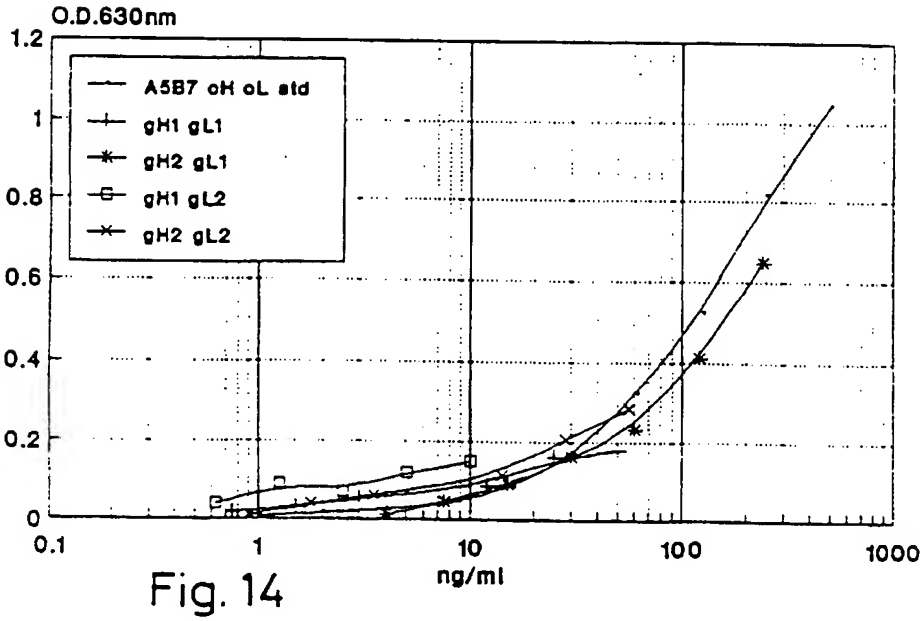

CEA Binding A5B7 gHgL
O.D.630nm
1.2
1
0.8
0.6
0.4
0.2
0
A5B7 cH cL std
gH1 gL1
gH2 gL1
gH1 gL2
gH2 gL2
0.1
1
10
100
1000
ng/ml

Fig. 14

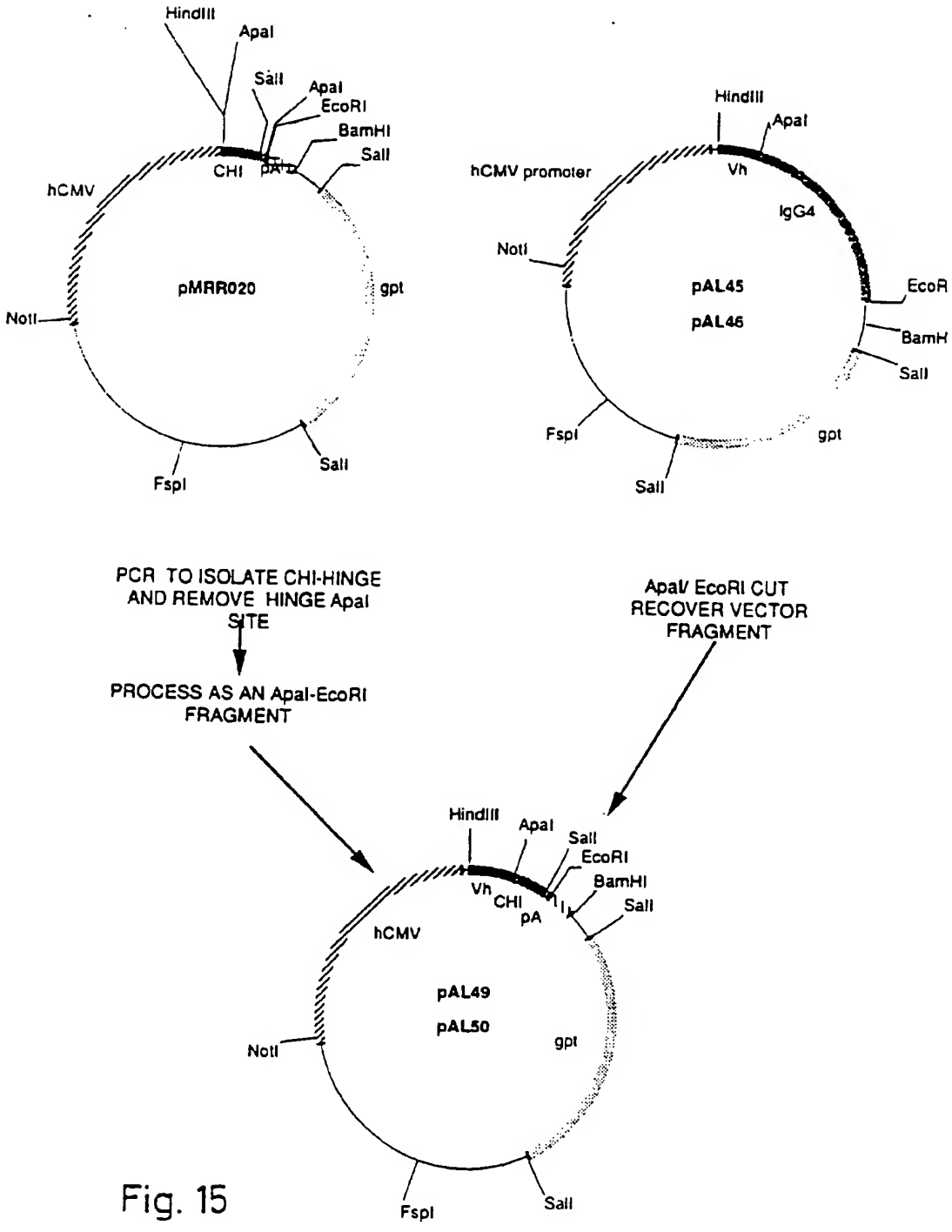


Fig. 15

VECTORS FOR gA5B7 Fab' EXPRESSION

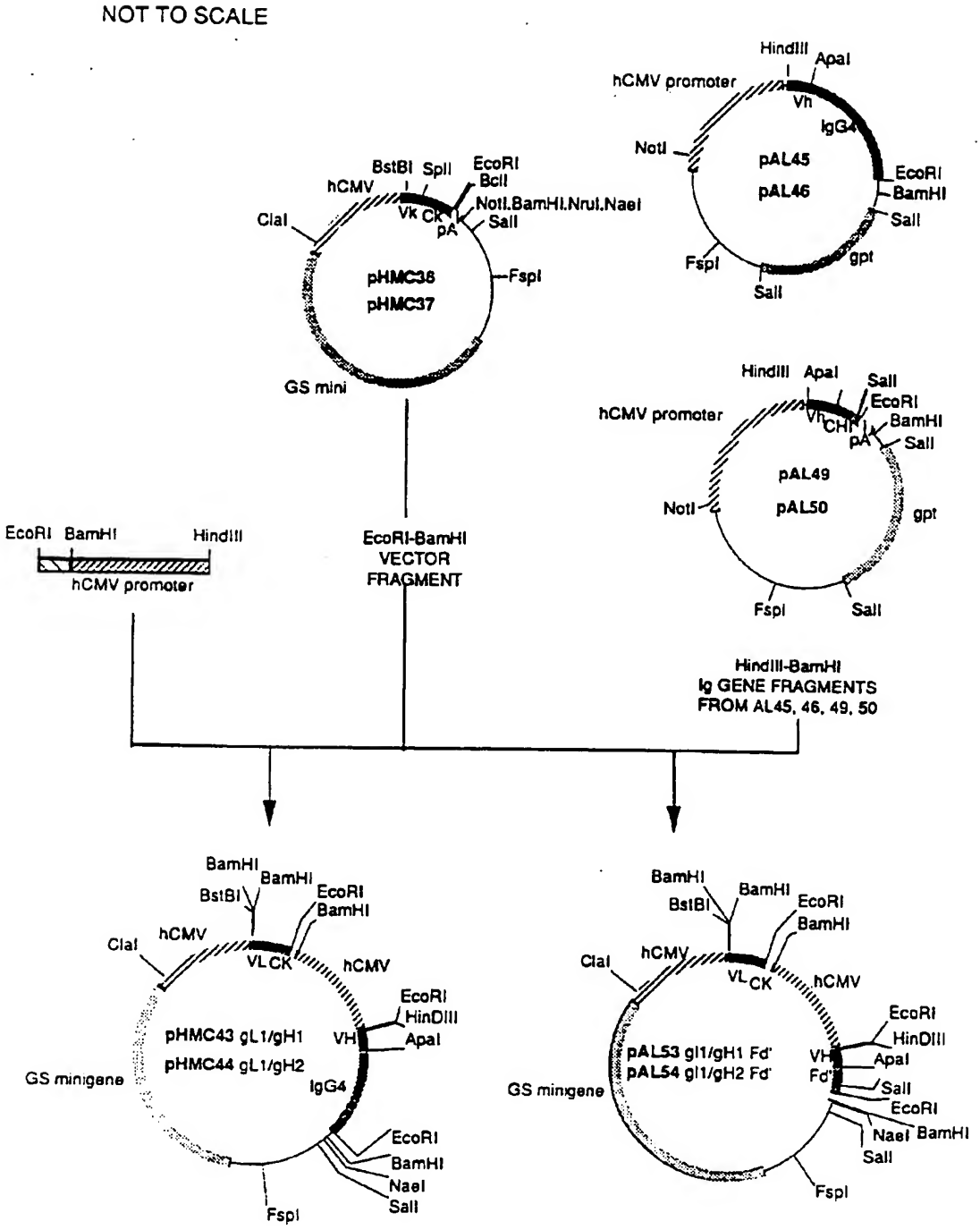
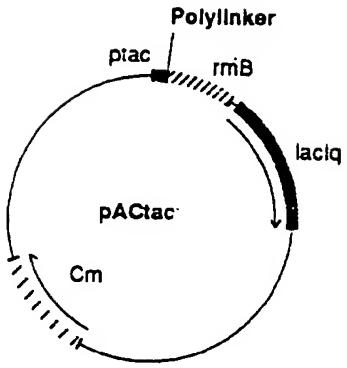
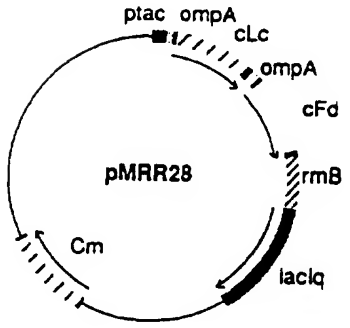


Fig. 16

VECTORS FOR gA5B7 CHO CELL EXPRESSION





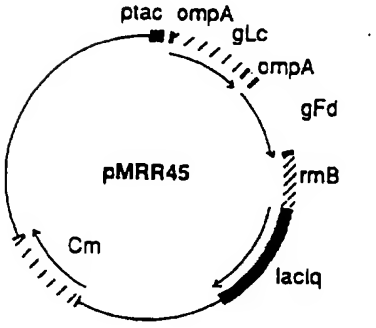


Fig. 17

Maps of A5B7 Fab' E.coli expression plasmids

CEA binding assay on E.coli supernatants containing A5B7 chimeric Fab'

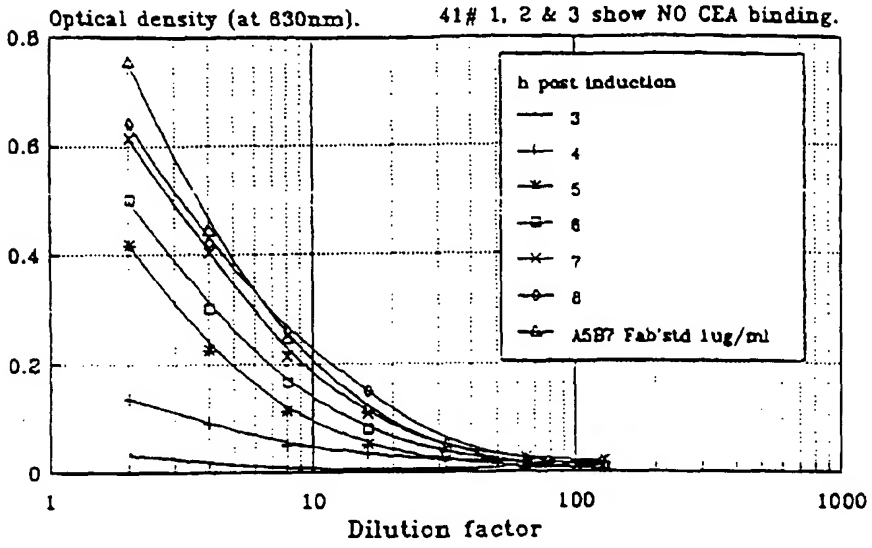


Fig. 18

CEA binding assay on E.coli supernatants containing A5B7 grafted Fab'

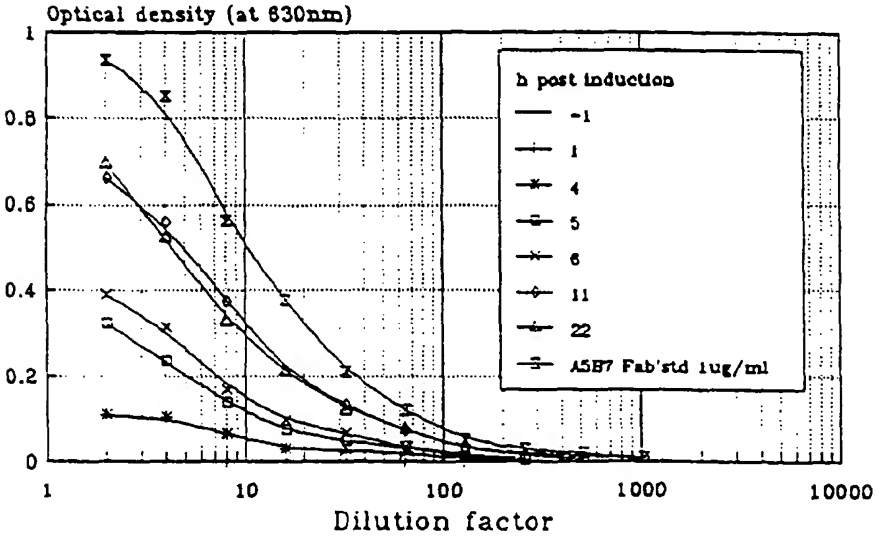


Fig. 19